National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE UNIVERSITY OF ALBERTA

SAMPLING AND MICROWAVE DISSOLUTION STUDIES FOR ELEMENTAL ANALYSIS OF SWAZILAND SOILS

(C)

by

SIPHO SAMUEL MDUDUZI MAMBA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

FALL 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-45544-6

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR SIPHO SAMUEL MDUDUZI MAMBA

TITLE OF THESIS SAMPLING AND MICROWAVE DISSOLUTION STUDIES FOR ELEMENTAL ANALYSIS OF SWAZILAND SOILS

DEGREE FOR WHICH THESIS WAS PRESENTED M.Sc.

YEAR THIS DEGREE GRANTED 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed) SMamba.

PERMANENT ADDRESS:

Department of Chemistry
University of Swaziland (UNISWA)
Private Bag
Kwaluseni Campus
Kingdom of Swaziland

DATED 10 - August 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled SAMPLING AND MICROWAVE DISSOLUTION STUDIES FOR ELEMENTAL ANALYSIS OF SWAZILAND SOILS

submitted by SIPHO SAMUEL MDUDUZI MAMBA

in partial fulfilment of the requirements for the degree of Master of Science.

Byron Kratochvil
Supervisor

DATE 28 July 88

THIS THESIS IS DEDICATED TO:

JAMES AND ASSIENAH

JABULANE, SIBUSISO, MKHULU AND NHLANHLA

ABSTRACT

Two adjacent sugarcane fields from Swaziland were sampled systematically in a grid pattern to study the variability of essential elements. Among the elements determinable by instrumental neutron activation analysis (INAA), Fe, Ti, Ca, Mg, Na, Al, Co, Cr, Sc, Eu, Dy, V, K, Ba, Ce, Cs, and Mn were found to be present at appreciable levels in both fields.

Graphite furnace atomic absorption spectroscopy (GFAAS) results for one field showed that lead concentrations were below 10 micrograms per gram of soil; therefore lead dioxide contamination of soil by automobile exhaust is not significant. Flame atomic absorption spectroscopy (FAAS) results showed that zinc is uniformly distributed over the entire field, whereas copper has significant spatial variability which follows a definite trend. Both elements are essential for sugarcane and their levels in the soil, on average, are 137 and 94 micrograms per gram for Cu and Zn respectively.

Conventional open-vessel dissolution procedures using HF, HNO_3 and $HClO_4$ are capable of achieving total decomposition of soil samples from Swaziland. However, explosion hazards and long dissolution times are encountered. A microwave dissolution procedure using HF and HNO_3 was found to achieve 95% sample dissolution when 500-mg test portions of soil were heated in a microwave

oven at 400 W for 7 minutes. Accurate results for zinc and copper were obtained by this method which was subsequently used in sample preparation.

To predict the number of samples to be taken from the population in order to achieve any level of precision, the student's t-test equation was found to be applicable within acceptable limits of error. When the standard deviation due to sampling is taken as the desired precision, the number of samples to be collected depends only on the tabulated value of t. With a sampling standard deviation of ±7% relative, the number of samples required for an equivalent precision was found to be 6 for zinc at the 95% confidence level. For copper, whose spatial variability is greater, the same number of samples yielded a precision of ±12% relative. Therefore in the design of sampling plans for multielement analyses, the minimum number of increments is dependent on the element which shows the greatest spatial variability.

ACKNOWLEDGEMENTS

My most sincere thanks go to Dr. B. Kratochvil for his valuable guidance during the course of this work and for his helpful suggestions in the preparation of this manuscript.

Thanks are also extended to George Dodsworth of the Royal Swaziland Sugar Corporation (RSSC), Simunye Sugar Estate for his permission to take samples from fields 201 and 202 on the estate, to David Nkonyane, B.Sc. Agr., for the systematic sampling patterns , to Norine Motkosky for her assistance with acid dissolution procedures, and to Annabelle Wiseman for the speed and accuracy exhibited in typing this thesis.

Financial support from the Canadian International Development Agency (CIDA), through the World University Service of Canada (WUSC) Special Programme for Swaziland, is gratefully acknowledged.

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
	1. Importance of Determining Trace Elements in Soil	1
	2. Theory of Sampling for Chemical Analysis	2
	2.1 Well-mixed Particulate Mixtures	4
	2.2 Gy's Approach for Heterogeneous Granular Materials	5
	2.3 Visman's Equations for Segregated Populations	7
	2.4 The Student's t-test Approach for Bulk Materials	8
	3. Analytical Methodology	8
	3.1 INAA: Theory, Applications and Limitations	9
	3.2 AAS: Theory, Applications and Limitations	10
	4. Goals	14
II.	COLLECTION OF SOIL SAMPLES FROM SWAZILAND	16
	1. Introduction	16
	2. Crop and Fertilizer History	16
	3. Experimental	20
	3.1 Equipment	20
	3.2 Mapping of Sampling Points	20

CHAPTER PAGE

3.3 Procedures for Collecting Samples..... 21

4. Sterilization of Soil Samples..... 23

III. PRELIMINARY STUDIES BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS..... 25

1. Introduction..... 25

2. Experimental..... 28

2.1 Selection of Samples for Preliminary Work..... 28

2.2 Preparation of Samples and Standards.... 29

2.3 Irradiation and Gamma Ray Spectrometry.. 30

3. Results and Discussion..... 34

3.1 Qualitative Analysis..... 34

3.2 Quantitative Analysis..... 35

4. Conclusions..... 48

IV. SAMPLE DISSOLUTION STUDIES FOR ATOMIC ABSORPTION SPECTROSCOPY..... 50

1. Introduction..... 50

2. Experimental..... 50

2.1 Mixing and Particle Size Reduction..... 50

2.2 Procedure for Obtaining Zinc and Copper Calibration Curves..... 51

2.3 Open-vessel Dissolution of Soil Using HNO_3, HF and $HClO_4$..... 54

2.4 Closed-vessel Microwave Leaching Using HNO_3..... 55

CHAPTER PAGE

2.5 Closed-vessel Microwave Acid Digestion Using HNO_3 and HF..... 56

2.6 Standard Additions and Atomic Absorption Measurements..... 56

3. Results and Discussion..... 57

3.1 Particle Size Distribution..... 57

3.2 Zinc and Copper Calibration Curves..... 59

3.3 Preliminary Estimates of Zn, Cu and Pb in Soil..... 66

3.4 Analytical/Measurement Uncertainties.... 69

3.5 Uncertainties Due to Subsampling..... 74

3.6 Validity of Analytical Procedures..... 76

3.7 Microwave Digestion..... 78

(a) Effect of acid mixture composition 78

(b) Effect of time..... 79

(c) Effect of microwave power..... 82

(d) Reliability of microwave acid dissolution..... 96

4. Conclusions..... 96

V. EVALUATION OF HETEROGENEITY OF THE SAMPLE SET AND DESIGN OF SAMPLING PLANS FOR TRACE ELEMENT ASSESSMENT IN SOILS..... 99

1. Introduction..... 99

2. Theory..... 99

CHAPTER PAGE

3. Experimental: Determination of Zinc and Copper in Soil Samples from Field Number 201..... 102

4. Results and Discussion..... 103

4.1 Quality Assurance..... 103

4.2 Trace Zinc and Copper Distribution in Field Number 201..... 107

4.3 Testing for Normality of Zinc and Copper Distributions in Field 201..... 113

4.4 Estimates of Increment Number Using the Student's t-test Equation..... 114

(a) Evaluation of uncertainties due to sampling..... 114

(b) Validity of the student's t-test equation for zinc..... 115

(c) Validity of the student's t-test equation for copper..... 116

4.5 Practical Considerations: Future Sampling Protocols..... 116

5. Conclusions..... 122

VI. SUGGESTIONS FOR FUTURE WORK..... 124

*****

REFERENCES..... 128

LIST OF TABLES

TABLE		PAGE
1.	Nitrogen, potassium, phosphorous, and zinc fertilizer application history for fields 201 and 202	19
2.	Irradiation, cooling and counting schemes for soil samples and standards	32
3.	Summary of observed peak energies for test portion from sample 201-03, obtained using irradiation scheme 1	36
4.	Summary of observed peak energies for test portion from sample 201-03, obtained using irradiation schemes 2 and 3	36
5.	Solid standards of elements identified in 6 samples from field numbers 201 and 202	39
6.	Liquid standards of elements identified in 6 samples from field numbers 201 and 202	40
7.	Summary of INAA results for NBS-1633a using long- and medium-lived isotope schemes	41
8.	Summary of INAA results for CCRMP SO-2 and SO-4 using the short-lived isotope scheme	42
9.	Results for minor and major elements in soil samples selected for preliminary work by INAA	46
10.	Results for trace elements in soil samples selected for preliminary studies by INAA	47

TABLE PAGE

11. Instrument parameters for flame AAS measurements..... 53

12. Particle size distribution for sample number 201-22..... 58

13. Average absorbance values for 0 to 10 ppm solutions of copper..... 60

14. Average absorbance values for 0 to 2 ppm solutions of zinc..... 63

15. Copper, zinc and lead determinations in 100 mg portions of soil sample 201-22..... 68

16. Total dissolution and standard addition results for a 500.21-mg test portion of sample 201-22..... 70

17. Copper and zinc concentrations in 4 test portions of sample 201-22..... 75

18. Estimation of subsampling varability in Zn and Cu concentrations in five samples of Swaziland soil digested with $HClO_4$..... 77

19. Zinc and copper in soil samples digested by microwave procedures..... 80

20. Effect of time on microwave digestion of soil samples from Swaziland..... 81

21. Microwave digestion of sample 201-24 at 35% power..... 83

22. Microwave digestion of sample 201-24 at 50% power..... 84

TABLE PAGE

23. Microwave digestion of sample 201-24 at 100% power..... 85

24. Comparison between referee method and microwave acid digestion at 50% power for 7 minutes..... 97

25. Values for zinc and copper after microwave digestion of sample 201-32 for quality assurance..... 106

26. Overall zinc and copper concentration values in soil samples from field number 201..... 108

27. Statistical summary of zinc and copper concentrations of 52 samples taken from field number 201..... 112

28. Results for 50 randomly selected groups of six samples used to test the validity of the student's t-test equation for zinc..... 117

29. Results for 50 randomly selected groups of six samples used to test the validity of the student's t-test equation for copper..... 119

LIST OF FIGURES

FIGURE		PAGE
1.	Plan of the Simunye Sugar Plantation, Simunye, Swaziland	17
2.	Sampling grid for field number 201	22
3.	Sampling grid for field number 202	24
4.	Schematic diagram of SLOWPOKE II reactor facility	31
5.	Diagram for high resolution gamma ray spectroscopy system	33
6.	Calibration curve for Cu^{2+}	61
7.	Calibration curve for Zn^{2+}	64
8.	Expanded linear region for Zn^{2+} curve	65
9.	Absorbance vs micrograms of spike in sample for Cu	71
10.	Absorbance vs micrograms of Zn^{2+} spike added	72
11.	Copper results vs time for microwave digestion at 35% power	86
12.	Zinc results vs time for microwave digestion at 35% power	87
13.	Copper results vs time for microwave digestion at 50% power	89
14.	Zinc results vs time for microwave digestion at 50% power	90

FIGURE PAGE

15. Copper results vs time for microwave digestion at 100% power..... 91

16. Zinc results vs time for microwave digestion at 100% power..... 92

17. Copper results vs time for microwave digestion at 35, 50 and 100% power..... 94

18. Zinc results vs time for microwave digestion at 35, 50 and 100% power..... 95

19. Control chart for zinc analyses of soil samples from field 201..... 104

20. Control chart for copper analyses of soil samples from field 201..... 105

21. Distribution pattern of zinc in field number 201..... 110

22. Distribution pattern of copper in field number 201..... 111

CHAPTER I

INTRODUCTION

1. Importance of Determining Trace Elements in Soil

Trace elements play a major role in the metabolic processes of biological systems. They were first recognized as a limiting factor in crop production in the 1920's [1,2]. The assessment of their levels in soil is important; distinct deficiency symptoms and lower yields are caused by very low levels while toxicity symptoms are caused by excessively high levels [1].

Copper, boron, iron, manganese, and zinc are essential to the sugarcane plant [3]. Boron is required for cell division, reproduction, translocation, and amino acid synthesis [1,3]. Copper is essential for the normal functioning of oxidase enzymes and also has an indirect effect on nodule formation [2]. Manganese is involved in photosynthesis and controls the start of several oxidation-reduction processes. Zinc is responsible for the biosynthesis of growth hormones such as indoleacetic acid (IAA). Iron is involved in the biosynthesis of chlorophyll.

Zinc has been found to be the only truly deficient element in Southern African soils [3,4]. Deficiency symptoms for iron have been reported in Swaziland, but they are only transient, i.e. high levels of calcium in the soil immobilize the iron so that it cannot be taken up by plants. This lack then appears as lime-induced chlorosis.

Standard agrochemical methods of analysis have been documented for soils [5-7]. Agronomists rely on chemical soil tests to evaluate the fertility status of soil. The main objective of soil testing is to obtain a value that will help to predict the amount of nutrients needed to supplement the supply in the soil under investigation, or to assess levels of elements that may interfere with proper plant growth.

2. Theory of Sampling for Chemical Analysis

Chemical analysis is a multistage process, which starts with primary sampling and ends with evaluation of the results [8]. Each stage contributes independently to the total overall error.

In accord with work by Kratochvil and Taylor [9], the total overall error, expressed as the variance, s_o^2, is related to the variance for the subsampling operation,

s_{ss}^2, to that of the sampling operation, s_s^2, and to that of the analytical measurement, s_a^2, by:

$$s_o^2 = s_a^2 + s_{ss}^2 + s_s^2 \quad (1)$$

Sampling involves identification and withdrawal of valid gross samples from the population. Equation (1) shows that regardless of the analytical precision, the soil test will be of little value if the test portion does not truly represent the population [10].

While a composite sample yields the average properties of the population at a minimum cost, it does not provide the analyst with population variability information [9,11] which is necessary for collaborative test samples and reference material usage. Since wide variability can occur in a field, analysts often face the problem of deciding what size the sample increment should be and the approximate number that have to be taken in order to obtain the desired precision of mean estimates. While Drees and Wilding emphasize that a large number of samples is necessary to achieve accurate population mean and variability estimates [12], there is a trade-off between precision and cost. Oversampling may be too costly while undersampling may not provide the desired

precision [13,14]. Consequently, the major goal of research on sampling over the years has been to derive equations which can be used to determine the minimum increment size and number necessary to obtain a given precision with a specified probability.

2.1 Well-mixed Particulate Mixtures

According to Benedetti-Pichler [15], a significant random sampling error may occur even in well-mixed populations. If a small number of particles is taken for analysis and the individual particles differ significantly in composition, the equation developed by Benedetti-Pichler:

$$n_p = \left[\frac{d_1 d_2}{\bar{d}^2}\right]^2 \left[\frac{100(P_1 - P_2)}{\sigma_s P_{ave}}\right](p)(1 - p) \qquad (2)$$

can be used to estimate the number of particles, n_p, required to hold the relative sampling standard deviation, σ_s, at a preselected level [16-18]. Here, d_1 and d_2 are the densities of type 1 and type 2 particles respectively in a 2-component mixture. P_1 and P_2 are the respective percentages of the sought-for substance in type 1 and type 2 particles. P_{ave} is the experimental average percentage of the sought-for substance in the mixture, and p is the fraction of type 1 particles in the bulk material.

Assuming that the particles are spheres with an average radius r and density $\bar{d}$, the optimum sample weight w is calculated using the equation

$$w = 4\pi r^3 n_p \bar{d}/3 \qquad (3)$$

For soils, this equation is useful only when subsampling a field sample that has been mixed, and when the particle size is relatively uniform and measurable.

2.2 Gy's Approach for Heterogeneous Granular Materials

According to Gy [19], the sample weight w required for any desired uncertainty level s_s^2 for a heterogeneous material containing a small quantity of the critical component is a function of particle shape, particle size distribution, the composition of the phases comprising the particulates, and the degree to which the critical component is liberated from the remainder of the material during particle size reduction by grinding. Gy defined the shape factor f as the ratio of the average volume of all particles having a maximum linear dimension equal to the mesh size of a screen to that of a cube which will just pass the same screen. The size range factor g is the ratio of the upper size limit (about 5% oversize) to the lower size limit (about 5% undersize). The liberation

factor l is the square root of the ratio of diameter of the average grains of sought-for component in the material to the diameter of the largest particles in the mixture. The composition factor c is given by

$$c = \left[\frac{1 - a}{a}\right] \left[(1 - a)\,\lambda_c + a\lambda_g\right] \qquad (4)$$

where a is the overall concentration of the mineral component of interest and λ_c and λ_g are densities of the critical component and the remainder of the bulk material. The sampling constant C_s, which is characteristic of the population under investigation, is given by

$$C_s = gclf \qquad (5)$$

and the minimum sample weight w necessary to represent a population characterized by a constant C_s and a maximum particle diameter d, when a fundamental variance s^2 is regarded as the maximum acceptable, is given by

$$w = \frac{C_s\ d^3}{s^2} \qquad (6)$$

Values for g, l and f can be easily estimated for soil.

However the composition factor c, which can take extreme values [19], cannot be easily estimated unless the physical mineralogical composition of the material is known. Gy's equation (6) involves the proportions of physical components which, in the case of soil, may not be easy to evaluate either because little is known about the mineralogy of the soil or because the sought-for substance is present in several minerals.

2.3 Visman's Equations for Segregated Populations

A potentially useful approach for estimating the size and number of field soil increments to collect is to use the Visman equation [9,16,20], which has been successfully applied to coal:

$$s_s^2 = \frac{A}{wn} + \frac{B}{n} \qquad (7)$$

Equation (7) describes sampling variance, s_s^2, as the sum of random (A) and segregation (B) terms. The constants A and B are a unique property of the population under investigation and can be experimentally determined [21]. Once A and B are known, the number of samples, n, of weight, w, that have to be taken to hold s_s at a preselected value at the desired confidence level can be calculated.

2.4 The Student's t-test Approach for Bulk Materials

If the sought-for substance follows a Gaussian distribution, the student's t-test equation from classical statistics [13]

$$n = \frac{t^2 s_s^2}{(\bar{x} - \mu)^2} \tag{8}$$

can be used. It is sometimes written as [9]:

$$n = \left(\frac{t^2 s_s^2}{R^2 \bar{x}^2}\right) 10^4 \tag{9}$$

where R is the acceptable relative standard deviation in percent and t is the student's t value, which depends on the degree of freedom of s_s^2 and the desired level of confidence. In equation (9) $\bar{x}$ and s_s^2 are estimates used to approximate the true population mean μ and variance σ^2 due to subsampling [9-11,13,14,21-24].

3. Analytical Methodology

The choice of analytical methods used in the sampling study was based on the availability of a SLOWPOKE II nuclear reactor facility [25,26] for instrumental neutron activation analysis (INAA) and a Varian Spectr AA-10 instrument for atomic absorption spectroscopy (AAS).

3.1 INAA: Theory, Applications and Limitations

The theory of INAA, a technique based on selectively inducing radioactivity in some of the atoms of elements making up the sample, and then selectively measuring the radiation emitted, is well developed [27-29]. Measurement systems for gamma radiation have undergone tremendous technological changes in recent years. Semiconductor detectors have replaced scintillation and gas-filled types. Lithium-drifted germanium and hyperpure germanium crystals with maximum impurity concentrations of less than 1×10^{10} cm^{-3} are currently being used [29].

The wide applicability of INAA stems from its sensitivity, selectivity and low limits of detection for many elements as compared to techniques like AAS, atomic emission spectroscopy, X-ray fluorescence, and anodic stripping voltammetry [27]. It is non-destructive, capable of multi-element analysis and requires minimal sample pretreatment. The applications of INAA in chemical analysis involve measuring impurities in semiconductors, characterization of standard reference materials such as coal, coal fly ash, soil, etc., and the determination of elements in tissues, soils and marine sediments [30-33]. The limitations of this technique also have been extensively investigated. Among the several sources of systematic error that are likely to occur in INAA, the

contribution of spectral and primary nuclear interferences are significant [34-36]. Spectral overlap causes erroneous results for zinc if europium and scandinum are present in large quantities [31]. The same error occurs for trace copper due to aluminum. Primary nuclear interferences arise from reactions induced by epithermal and fast neutrons on elements other than those to be measured, but yielding the same product-nuclide as the (n, γ) reaction. Theoretically calculated and experimentally determined interference factors can be used to reduce this error [34-36]. Also, INAA cannot be used for the determination of elements that do not react with neutrons to form radioactive elements in adequate quantities or with appropriate halflives.

3.2 AAS: Theory, Applications and Limitations

Among the elements not determinable at trace levels in soils by INAA are cadmium and lead [37]. Also, as mentioned previously, with this technique, copper and zinc determinations in soil may suffer from spectral interferences which tend to bias results in one direction. Atomic absorption spectroscopy (AAS) on the other hand provides a sensitive means for the determination of these elements with detection limits of about 1 ppm or less. Up to 0.2% relative analytical

standard deviation is is presently achievable [37-39]. In the analysis of soil for Zn, Cu and Pb, AAS is most likely to be the complementary method of choice.

The theory of AAS, a technique based on the selective absorption of narrow emission lines from a hollow-cathode lamp by unexcited and unionized ground state atoms in a flame, is well developed [37,40]. The superior selectivity of AAS is due to the use of a radiation source that emits a line of the same wavelength as the one to be used for the absorption analysis [41].

Several different types of interferences can occur in flame atomic absorption spectroscopy. Chemical interference is not likely to cause errors in Cd, Cu and Zn determinations in the air-acetylene flame. In the case of lead, a number of anionic interferences have been reported. These can be largely overcome by addition of EDTA [42]. Background absorption, which affects Zn and Pb, can be corrected for by using a deuterium continuum source. Since all four elements have significantly high ionization potentials, ionization interference is minimal, and physical interferences such as matrix effects can be corrected for by the method of standard additions.

Unlike INAA, where sample pretreatment often is not necessary, sample dissolution which is time consuming, must precede AAS measurement. Open vessel acid attack on

samples by the use of HF, $HClO_4$, HNO_3, H_2SO_4, and HCl, either individually or in combination, is well documented. While HNO_3 alone may be sufficient to liberate metals which are bound to organic matrices, Jackson and Newman [43] warn against its use for inorganics. Unless HF and hot, concentrated $HClO_4$ are used, metals contained in crystal lattices of unweathered primary minerals will resist acid digestions. In a study of the Canadian reference soils CCRMP SO-1 through SO-4 using aqua regia [44], the poor extraction of Pb, Al and Fe was attributed to the presence of complex alumino silicates. Minerals expected to follow this trend are: quartz (SiO_2) for Si, alumina (α-Al_2O_3) for Al, cassiterite (SnO_2) for Sn, magnetite (Fe^{2+}-$Fe_2^{3+}O_4$) for Fe, chromite ($FeCr_2O_4$) for Cr, zircon ($ZrSiO_4$) for Zr and rutile (TiO_2) for Ti [45].

An open-vessel dissolution technique employing a mixture of HNO_3, HF and $HClO_4$ has been found to achieve 100% dissolution of soils [46]. The HNO_3 serves to destroy the bulk of the organics while the $HClO_4$ is still cold and nonoxidizing [47]:

$$(CH_2)_n + 2HNO_3 \rightarrow CO_2 + 2H_2O + 2NO \qquad (10)$$

After reaction (10) is complete, the mixture is warmed enough for HF and $HClO_4$ to start attacking the inorganics. Complete dissolution occurs when samples are fumed at 200°C for 10-12 h. This time constraint is one disadvantage with the method. Another is the explosion hazard when hot, concentrated $HClO_4$ comes into contact with oxidizable substances. The dangers associated with the use of $HClO_4$ are well known [48]. Esters of perchloric acid are shock-sensitive and may spontaneously explode upon standing at room temperature. Constant supervision and installation of a special all-steel fume hood equipped with wash down facilities is therefore necessary.

Reports on the first analytical application of microwave ovens appeared in 1975 after successful dissolution of organics in a biological sample [49]. Recently, Nadkarni [50] reported that rock samples, which normally took 5 days to dissolve using open-vessel methods, took only 3 minutes by closed-vessel microwave techniques. Teflon PFA vessels manufactured by Savillex Corporation are widely used for microwave dissolution [47,51-55] and have been found to be better suited to microwave work than polycarbonate [55] or Parr Teflon vessels [50]. Among the several types of Teflon PFA vessels currently available, Berman and co-workers

recommend the completely closed type (CCT) for samples containing small quantities of organic matter [52].

The success of closed-vessel microwave dissolution over conventional open vessel techniques is due to the physical processes occurring inside the vessels. There is on-going speculation about the actual mechanism but all evidence attributes the rapid heating to the instant uptake of energy by the whole sample [53]. This is in addition to the slow heat transfer which occurs via molecular collisions in open-vessel techniques. Adsorptive polarization, a physical process that occurs when dipoles continuously and rapidly align when exposed to an oscillating field of appropriate energy is also known to cause mechanical stress within the sample producing agitation and rupture of sample surfaces. This rapidly exposes fresh surfaces to the attacking acid. Kingston and Jassie [47] have recently shown that the increased pressure inside the vessel causes the acids to boil at temperatures far above their normal boiling points. Since dissolution involves chemical reactions between acid and sample surfaces, the elevated temperatures play a significant role in the overall process.

4. Goals

The goal of this study was to study the trace element composition of a series of samples collected from one or two appropriate fields. Two elements were selected for detailed investigation of their distributions. With this population information a statistical evaluation was performed to estimate the number of samples required to measure element concentrations to a given confidence level.

In addition to the sampling study, improvements in the sample preparation steps were also investigated. Optimum conditions for microwave dissolution procedures, previously used successfully in this laboratory on biological materials, were developed for the soils used in this project.

CHAPTER 2

COLLECTION OF SOIL SAMPLES FROM SWAZILAND

1. Introduction

A total of 94 soil samples were collected from sugarcane fields in Swaziland. Two adjacent fields, used to grow the NCo-376 variety of *Saccharum barberis*, were sampled in a systematic grid pattern with a random start point. Permission to take samples was kindly given by the Simunye Sugar Plantation of the Royal Swaziland Sugar Corporation Ltd. The importation of samples into Canada was made possible through Food Production and Inspection Permit No. 65260 issued by the Edmonton section of Agriculture Canada.

2. Crop and Fertilizer History

The initial clearing of indigenous bush vegetation was done in 1978. Ploughing and planting of the first sugarcane stalks on field numbers 201 and 202 (Figure 1) took place in November of the same year. The first harvest occurred during the winter of 1981. At the time of sampling, the sixth harvest had just been completed.

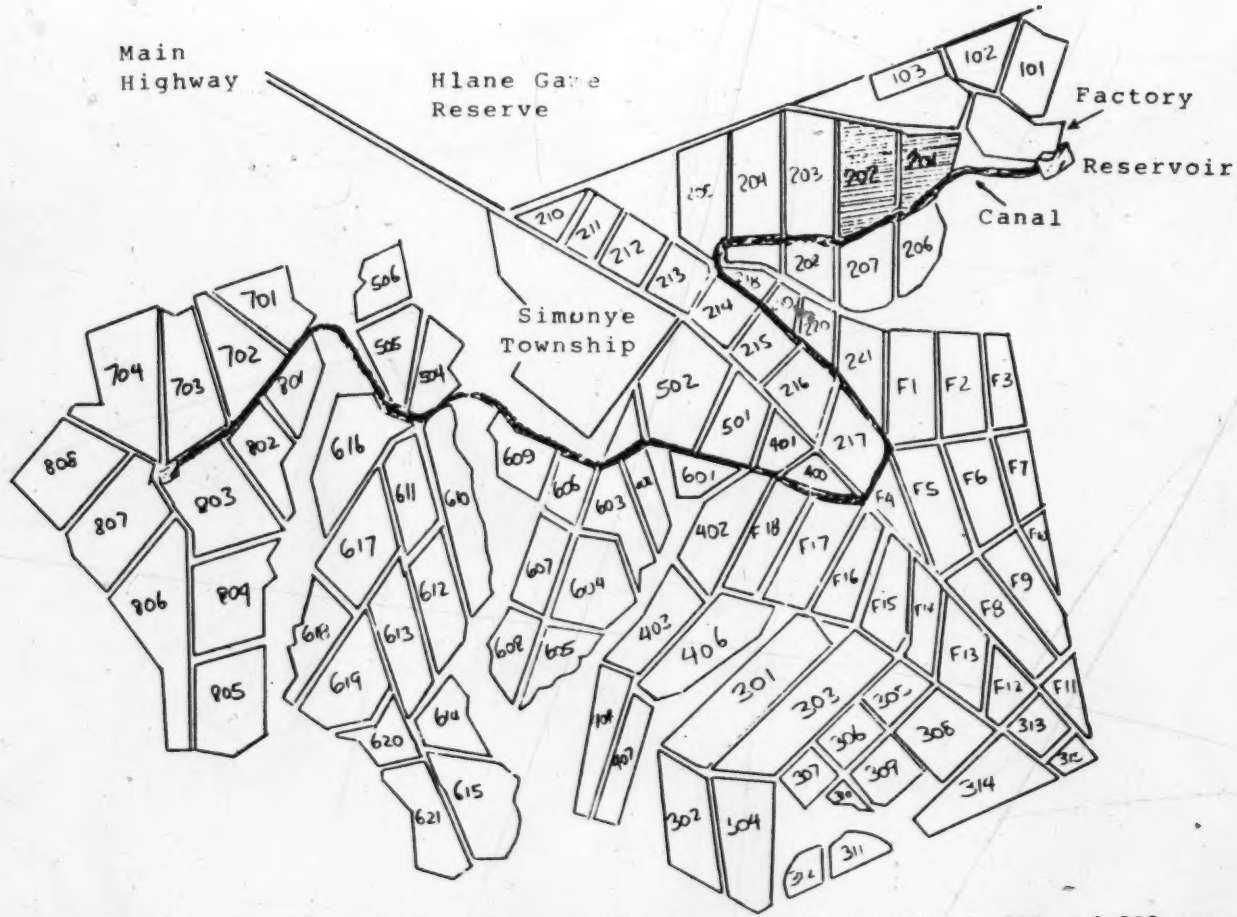

Figure 1. Plan of the Simunye Sugar Plantation, Simunye, Swaziland. Sampled fields 201 and 202 are shown as shaded areas in upper right. Scale is approximately 1 cm = 500 m. Meandering dark line is irrigation canal.

The appearance of zinc deficiency symptoms in the initial crop led to a direct application of $ZnCl_2$ pellets in the form of a soil dressing in 1982. Iron deficiency symptoms have also been reported but fertilizers containing Fe have not been applied. Even though the soils in Swaziland have a high Fe content, chlorosis is a typical iron-deficiency symptom observed in the sugarcane plants. This is probably due to the high calcium content of the soil. Calcium is added in the form of lime to raise the soil pH to about 6.5. The solubility of iron in soils is pH dependent; insoluble hydroxides of iron are formed at a pH of 6 and above [56] thereby reducing the likelihood of Fe being available to the plants. This triggers lime-induced chlorosis which can be remedied by adding acidulents to the soil [57].

Boron, manganese and copper deficiency symptoms have not been reported on these soils, therefore there has been no supplementing of the naturally occurring sources of these nutrients. A summary of zinc soil dressings is given in Table 1.

Sugarcane also requires nitrogen, phosphorous and potassium in large quantities. The commercial nitrogen carriers are: MAP (monoammonium phosphate, 12% nitrogen), DAP (diammonium phosphate, 21% nitrogen), SAAIFOS (3.8% nitrogen), and urea (47% nitrogen). The phosphorous

Table 1. Nitrogen, phosphorous, potassium, and zinc application history for fields 201 and 202.

Period	N	P	K	Zn
		Field 201		
1978	46	55[b]	126	0
1981	138	40[b]	83	0
1982	0	0	0	2
1983	128	39[c]	89	3
1984	169	39	0	0
1985	183	39	0	0
1986	172[a]	43	0	0
		Field 202		
1978	37	39[b]	88	0
1981	108	10[b]	0	0
1982	51	30[c]	83	2
1983	128	39[c]	89	3
1984	170	39	0	0
1985	183	39	0	0
1986	170[a]	43	0	0

Values are reported as kg of carrier added to the entire field except Zn (kg carrier/ha).

N carrier was urea except a = $(NH_4)_2SO_4$.

P carrier was DAP except b = MAP and c = SAAIFOS.

carriers are: MAP (52% phosphorous), DAP (54% phosphorous) and SAAIFOS (12% phosphorous). Pure KCl pellets, containing 52% potassium are used as K carriers. A summary of N, P and K fertilizr applications on fields 201 and 202 is given in Table 1.

3. Experimental

3.1 Equipment

A 10-cm hard ground steel sampling auger (Edelman Eijkelkamp, Holland) was used. Polyethylene bags (10 cm × 10 cm) were used as containers. Labels and rubber bands were also provided for sample identification and closure. Walking paces were used to estimate distances between sampling points.

3.2 Mapping of Sampling Points

The location of sugarcane fields at the ranch is shown in Figure 1. The factory, where cane sugar (sucrose) and molasses are produced by the action of evaporation, seeding and centrifugation on juice extracted from crushed cane, is situated 2 miles north-east of the town. Field numbers 201 and 202, shown as shaded areas next to the factory, had just been harvested at the time of sampling.

The northern boundary of field 201 is marked by a

road which links the factory to the town while the southern boundary is marked by a by-pass road from the irrigation reservoir which follows the canal to the main highway. The eastern boundary is marked by a fence which separates field 201 from the factory.

Similarly, the northern and southern boundaries of field 202 are an extension of the same in the westerly direction. The western boundary is marked by the eastern boundary of field 203 while the road separating field 201 from field 202 is taken as the eastern boundary.

3.3 Procedures for Collecting Samples

The top left corner of field 201, and 10 paces towards the interior of the field along irrigation valve 149, as shown in Figure 2, was taken as the random start point. At a measured average pace size of 80 cm, the first augerful of soil was taken 8 m from valve 149. This sampling point was designated 201-01 and the sample was placed inside an appropriately labelled polyethylene bag. Sample 201-02 was taken 40 m away from 201-01 along the same lateral. So were all samples through 201-11 at the end of the lateral. Sample 201-12 from the next lateral was taken perpendicular to 201-11 and additional samples taken at 40 m intervals towards valve 148. Similarly, sample 201-23 was taken 8 m from valve 147 and

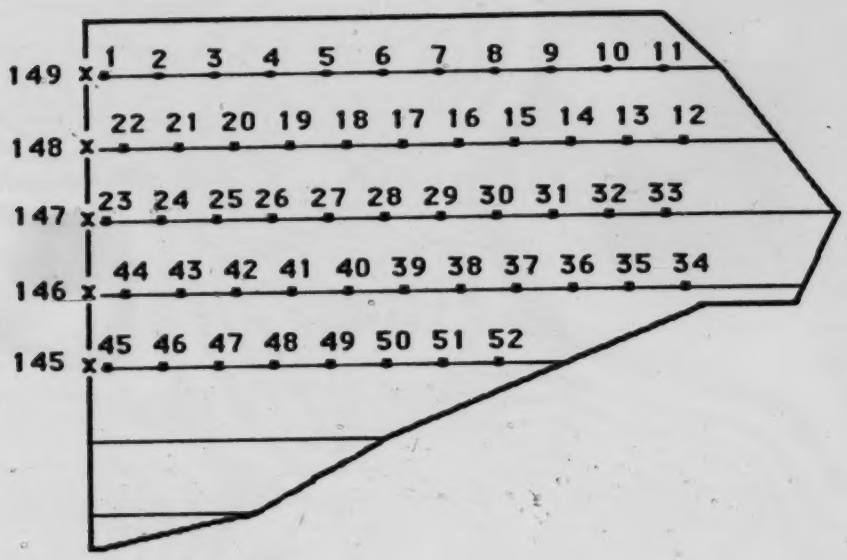

Figure 2. Sampling grid for field number 201. Drawn to a scale of 1 cm:50 metres and covers a total area of 17 ha. Number beside x refers to irrigation sprinkler valve used to supply water to lateral.

samples 201-24 through 201-33 were taken at 40 m intervals along this lateral. Samples 201-34 through 201-44 were taken towards valve 146 while 201-45 through 201-52 were taken from the valve 145 lateral.

Sample 202-01 was taken by measuring 20 paces towards the interior of field 202 from valve 143. Samples 202-02 through 202-06 were taken along this lateral at 90 m intervals as shown in Figure 3. Returning to valve 144, samples 202-07 through 202-12 were taken in a similar fashion. In total, 42 samples were collected from field 202, and each one of them was a composite of 2 augerfuls of soil.

4. Sterilization of Soil Samples

To satisfy importation requirements for soil samples into Canada, the samples were transferred to glass beakers, spread in a thickness of approximately 5 centimeters and heated in a forced-air oven (Gallenkamp) at 120° for 6 days. Samples were then transferred to cleaned new polyethylene bottles and packed for shipment.

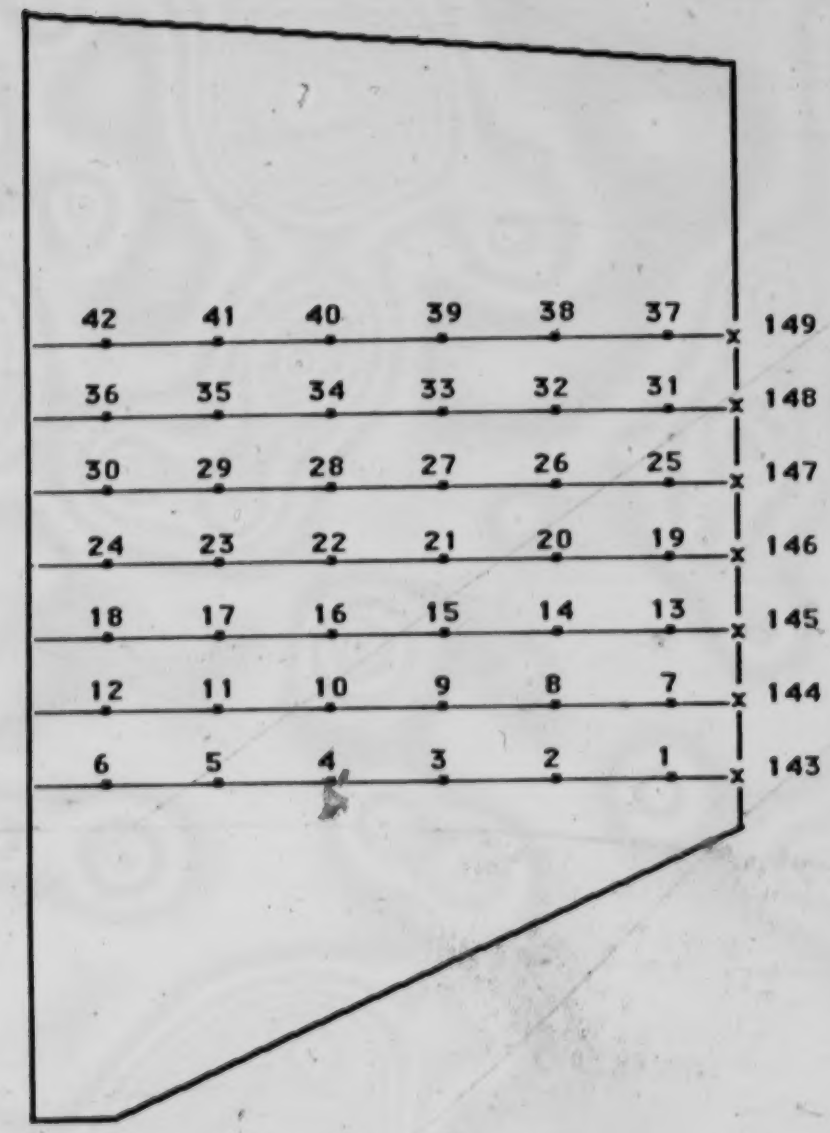

Figure 3. Sampling grid for field number 202. Drawn to a scale of 1 cm:50 m and covers a total area of 37 ha. Number beside x refers to irrigation sprinkler valve used to supply water to lateral.

CHAPTER 3

PRELIMINARY STUDIES BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

1. Introduction

Because the soils sampled in this study are uncharacterized, preliminary work was necessary to determine which essential and toxic elements were present, and in what approximate quantities. On the basis of time, cost and convenience, instrumental neutron activation analysis (INAA) was found to be a suitable technique for this purpose. It frequently reqûires little beyond weighing and encapsulation for sample preparation. INAA is also an excellent technique for multielement analysis on a single test portion of sample [27,29].

When a sample is bombarded with neutrons, radioactivity is induced, i.e. radioactive isotopes of stable nuclei are produced. The activity of a radioisotope at a given time, A_t, can be expressed in terms of the saturation activity, A_{sat}, which is the activity obtained by prolonged activation ($t \to \infty$), activation time, t, and the nuclide-dependent radioactive decay constant, λ, by:

$$A_t = A_{sat}(1 - e^{-\lambda t}) \qquad (11)$$

Equation (11) shows that as activation proceeds, there is an increase in activity, but the rate of growth of activity decreases [29]. This limits useful irradiation times to about one half-life, $t_{1/2}$, of the element of interest. This value is related to the first-order rate constant λ by:

$$t_{1/2} = \frac{\ln 2}{\lambda} \qquad (12)$$

Equation (12) provides the theoretical basis for irradiation schemes employed in INAA work [33]. Short-lived isotopes are normally irradiated in the SLOWPOKE reactor used in this work for 30 to 120 s while medium and long-lived isotopes require 2 h or longer, depending on the nature of the sample being investigated.

The analytical schemes also include cooling and counting times. These are chosen to maximize the activity of radioisotopes of interest while at the same time minimizing the activity of other radioisotopes, especially potential spectral interferents.

Modern advances in computer technology have resulted

in complete automation of gamma ray spectrum acquisition [29]. Off-line computers allow computations in batch mode using programs which can be written and optimized for specific applications. Commonly used routines recognize individual peaks in the spectrum, separate the energy area of interest, A, from the background area, B, by curve fitting and calculate the uncertainty in counting statistics at the 95% confidence level by:

$$\% \text{ error} = \left(\frac{1.96\ \sqrt{A + 2B}}{A}\right) 100 \qquad (13)$$

Being a comparative analytical technique, quantitative analysis in INAA is based on the equation

$$\frac{W_{e,sa}}{W_{e,st}} = \frac{A_{i,sa}}{A_{i,st}} \qquad (14)$$

where $W_{e,sa}$ is the weight of the element being determined in the test portion, $W_{e,st}$ is the weight of that element in a standard test portion, $A_{i,sa}$ is the activity of radionuclide i in the test portion and $A_{i,st}$ is the activity of that radionuclide in the standard test portion.

When correcting for systematic errors introduced by primary nuclear interferences, interference factors are

used. The interference factor, F, is the weight, in micrograms, of the interfering element which produces the same activity of the radioisotope used for analysis as 1 μg of the element of interest via the (n,γ) reaction [34]. The correction is done by analysing for the element of interest and the interfering element, then dividing the concentration of interfering element by the interference factor to yield the amount to be subtracted from the measured concentration of the element of interest. Consequently, the smaller the value of F, the bigger the contribution of nuclear interference to the total overall error.

2. Experimental

2.1 Selection of Samples for Preliminary Work

Three samples from each of the two fields were selected on the basis of both randomization and common-sense judgment. A table of random numbers was used [58] but samples picked from adjacent points on the map were not considered, in an attempt to make all parts of the population fairly represented in the limited number of analyses that were done in this preliminary study. Samples 201-03, 201-32, 201-48, 202-07, 202-17, and 202-39 were chosen for INAA.

2.2 Preparation of Samples and Standards

Whole samples were hand-ground to a very fine powder using an agate mortar and pestle. The samples were then tumbled end-over-end for 2 h. Portions were removed, placed in clean, glass vials and dried for 16 h at 110°C in an oven (Cenco Instrument Corporation, Model R).

Approximately 350-mg test portions were packed into nitric acid washed 400 µL polyethylene micro-centrifuge tubes. The tubes were heat-sealed and enclosed in an appropriately labelled second larger polyethylene vial for irradiation.

Liquid standards were obtained from commercial atomic absorption grade solutions (Fisher Scientific Company, Aldrich Chemical Company, Spex) by pipetting the appropriate quantities of stock solutions, using Eppendorf micropipets with disposable plastic tips, directly into polyethylene micro-centrifuge tubes in the same manner as the samples. Solutions were irradiated as is, i.e. without drying. Solid standards were obtained from Fisher Scientific and Aldrich Chemical Company. Standard reference materials used were NBS-1633a, coal fly ash (National Bureau of Standards, Washington, DC), CCRMP-SO-2 and CCRMP-SO-4 soil (Canadian Certified Reference Materials, CANMET, Ottawa, Ontario).

2.3 Irradiation and Gamma Ray Spectrometry

The irradiation and counting operations were carried out at the nuclear reactor facility located in the Dentistry-Pharmacy building at the University of Alberta. The SLOWPOKE II reactor, developed by Atomic Energy of Canada Limited (AECL) at Chalk River Nuclear Laboratories, is a small pool-type nuclear reactor using fully enriched uranium fuel as a source of neutrons. Thermal fluxes of 0.5×10^{11} to 1.0×10^{12} are obtainable at 20 kW of maximum thermal power [25]. The core, which provides a reliable source of neutrons, consists of 300 fuel elements arranged in concentric circles. These elements are made of an Al-U alloy in which the uranium is enriched to 93% ^{235}U and has a critical mass of only 850 g. The low critical mass, combined with the large negative temperature coefficient of reactivity is a novel feature of the SLOWPOKE II [26].

All samples were loaded at the station and rapidly transferred to the reactor core via pneumatic tubes shown in Figure 4. Based on prior experience with geological materials, the irradiation schemes shown in Table 2 were employed.

All counting was carried out using the high resolution gamma ray spectroscopy set-up shown in Figure 5. An Ortec coaxial HP(Ge) detector, cooled to liquid

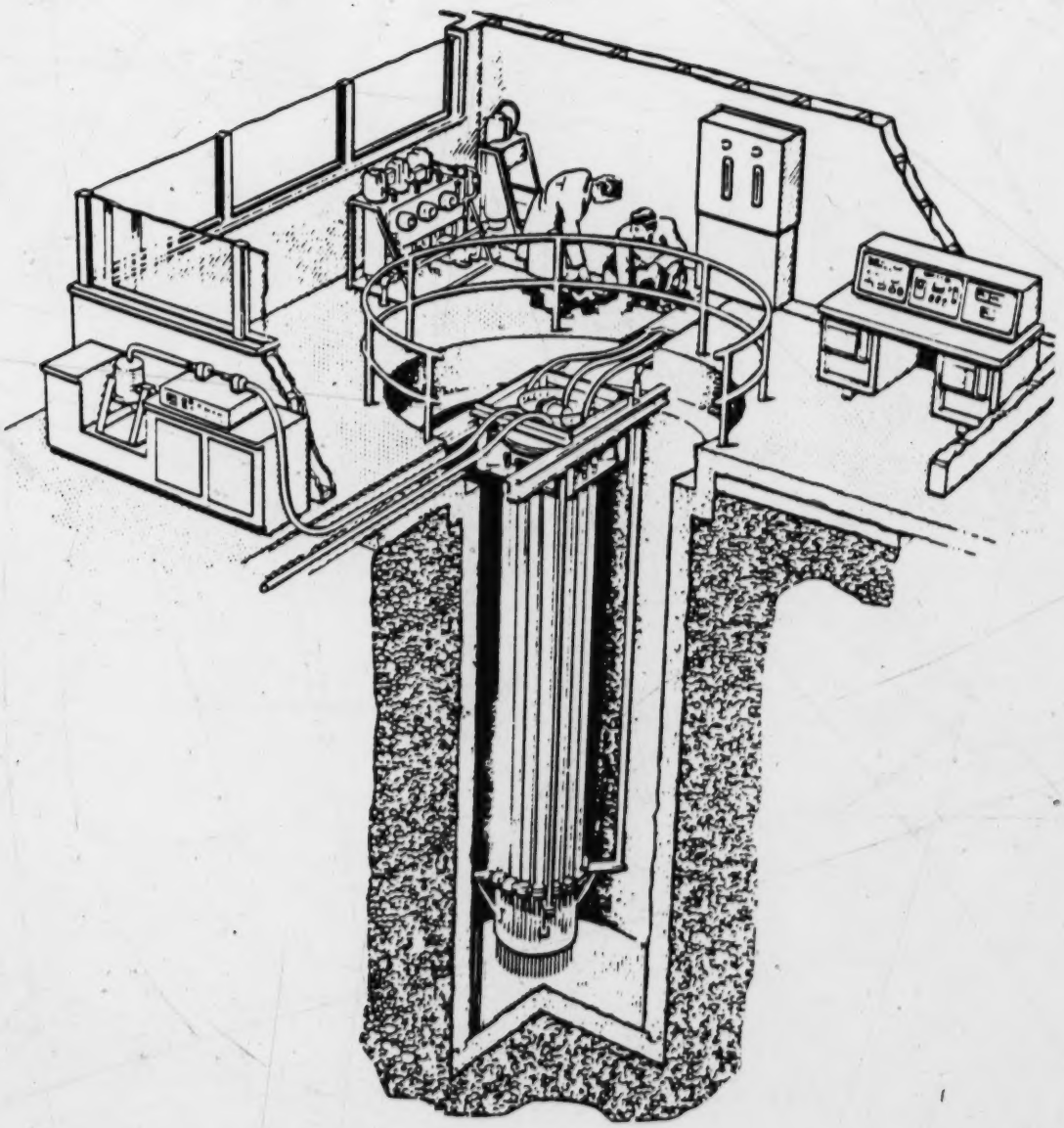

Figure 4. Schematic diagram of SLOWPOKE II reactor facility (Ref. 25).

Table 2. Irradiation, cooling and counting schemes for soil samples and standards. t_{irr} refers to the time of exposure to neutrons, t_{cool} refers to the time samples were required to decay after activation, t_{count} refers to the time required to acquire the gamma ray spectrum. All times in minutes.

Isotope type	t_{irr} (m)	t_{cool} (m)	t_{count} (m)	Neutron Flux ($ncm^{-1}s^{-1}$)	Counting Geometry (cm from sample to detection)
short-lived	2	.25	10	1.0×10^{11}	6
medium-lived	120	120	60	1.0×10^{12}	12
long-lived	120	372	600	1.0×10^{12}	1

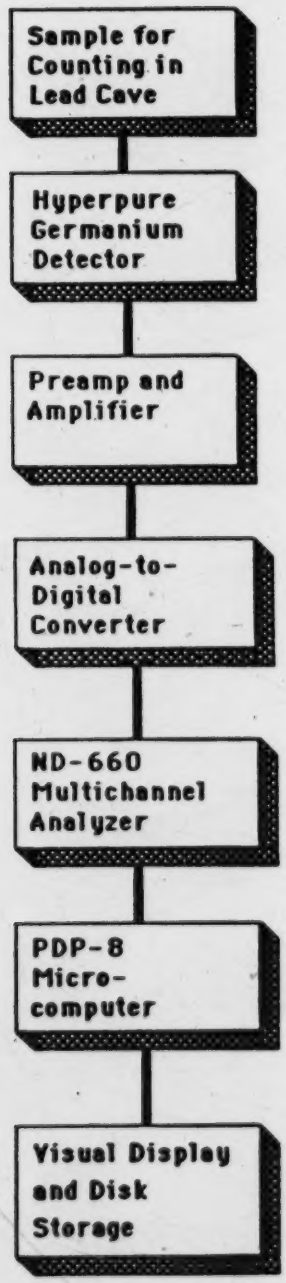

Figure 5. Diagram for high resolution Gamma Ray Spectroscopy system.

nitrogen temperatures through the use of a Dewar flask, was used. The detector has 10% relative efficiency, a measured full width at half maximum (f.w.h.m.) of 2.0 keV, and a peak-to-Compton ratio of 36:1 for the 1332 keV photo peak of ^{60}Co. It is coupled to a Nuclear Data (ND) 660 multichannel analyzer (MCA) with EG&G Ortec 572 preamplifier and amplifier stages. Analog signals are fed to the next stage, the ND575 ADC, and the resulting spectrum, which spans an energy range of 40-3200 keV, is stored on floppy disks in binary form. For calibration of the energy scale, the 88.037 keV ^{109}Cd line (on the low energy side) and the 1332 keV ^{60}Co line (on the high energy side) were used. Qualitative and quantitative computations were carried out off-line in batch mode using standard Nuclear Data software (with modifications made by personnel at the SLOWPOKE II facility) and a PDP/11 minicomputer (DEC). Primary nuclear interferences were corrected for manually using the method described earlier (Section 1) and values for the interference factors listed in reference 34.

3. Results and Discussion

3.1 Qualitative Analysis

A summary of the analytical results obtained in a

353.44 mg test portion of sample 201-03 is shown in Table 3 for short-lived isotopes and in Table 4 for medium and long-lived isotopes. The literature peak energies were obtained from standard INAA tables [28]. Most of the essential elements described earlier are present. Similar results were obtained for all 6 samples irradiated.

3.2 Quantitative Analysis

The quantities of solid standards irradiated under the same conditions as the samples are shown in Table 5 and those of liquid standards are shown in Table 6.

Results for certified reference materials are shown in Table 7 for NBS 1633a - Coal Fly Ash (U.S. National Bureau of Standards, Washington, DC), and Table 8 for CCRMP SO-2, a ferro-humic podzol soil, and CCRMP SO-4, a black chernozemic soil (Canadian Certified Reference Materials Project, Soil Research Institute, Central Experimental Farm, Ottawa, Ontario).

Even though the coal fly ash matrix does not match the matrix of the soil samples, the results of NBS-1633a can be used to estimate the validity of the method, the long-lived isotope scheme, and the library of standards because INAA is considered to be a relatively matrix-independent technique. Similarly, CCRMP SO-2 and SO-4 results can be used for the short-lived isotope scheme and

Table 3. Summary of observed peak energies for test portion from sample 201-03, obtained using irradiation scheme 1.

Observed peak energy (keV)	Probable isotope	Literature peak energy (keV)
443	^{128}I	443*
743		744
847	^{56}Mn	847
1812		1811*
2115		2113
1435	^{52}V	1434*
1780	^{28}Al	1779*
3086	^{49}Ca	3084*
1369	^{24}Na	1368*
2756		2754
320	^{51}Ti	320*
1015	^{27}Mg	1014*
93	^{165}Dy	95
362		362*
1041	^{66}Cu	1039*

*γ-ray energy normally used for analysis.

Table 4. Summary of observed peak energies for test portion from sample 201-03, obtained using irradiation schemes 2 and 3.

Observed peak energy (keV)	Probable isotope	Literature peak energy (keV)
1525		1525*
312	^{42}K	313
103		103*
70	^{153}Sm	70
1596		1595
487		487*
816		816
329	^{140}La	329
560		559*
868	^{76}As	868
776		777
1318		1317
1044		1044
1475		1475
554	^{82}Br	554*
293	^{143}Ce	293*
1099		1099*
1291		1292
192		192
142	^{59}Fe	143
123		124
216		216
133		134
496		496*
373	^{131}Ba	373
811	^{58}Co	810*
208		208*
113		113
249	^{177}Lu	250
91		91
531		531*
320		319
411	^{147}Nd	410

(Continued)

Table 4. (Continued)

Observed peak energy (keV)	Probable isotope	Literature peak energy (keV)
482		482*
133		133
346		346
136	^{181}Hf	136
889		889*
1120	^{46}Sc	1120
320	^{51}Cr	320*
344		344
122		122
964		964
1086		1086
1408	^{152}Eu	1408*
605		605
796		796*
569		569
1364	^{134}Cs	1365
1115	^{65}Zn	1115*
1077	^{86}Rb	1077*
1173	^{60}Co	1173*
1332		1332
1120		1121
1221		1222*
67		68
1231	^{182}Ta	1231
312		312*
299		300
416		416
86	^{233}Pa	87
514	^{85}Sr	514*
411	^{198}Au	412*
142		141*
779	^{99}Mo	778

*γ-ray energy normally used for analysis.

Table 5. Solid standards of elements identified in the 6 samples from field numbers 201 and 202.

Solid standard*	Element of interest	Weight of standard (mg)	Weight of element of interest (μg)
$Al_2(SO_4)_3$	Al	147.5	23,260
$CaCO_3$	Ca	121.3	48,574
Na_2CO_3	Na	16.7	7,244
K_2CO_3	K	19.8	11,203

*All standards were dried for 8 h at 110°C prior to encapsulation.

Table 6. Liquid standards of elements identified in 6 samples from fields 201 and 202. Volumes are given in μL, concentrations in ppm and weights in μg units.

Element	Concentration of stock solution, μg/μL	Volume used μL	Weight of element, μg
Fe	1004	350	351.40
Mo	1019	10	10.19
Hf	100	30	3.00
Ce	1002	60	60.12
Cr	996	20	19.62
Eu	49.35	20	0.987
Cs	200.6	30	6.018
Zn	998	70	69.86
Rb	1002	30	30.06
Co	100	20	2.00
Ta	10	30	0.30
Sb	50	20	1.00
Sc	49.6	20	0.992
Th	995	5	4.975
Ni	994	160	159.04
Ba	1000	200	200
Br	11,797	15	177.075
Sm	199.6	20	3.992
La	1003	20	20.16
As	1000	20	20
Lu	10.22	20	0.2044
Na	994	40	40.24
Mn	1012	75	75.90
V	1000	400	400
Ti	1000	75	75
Cu	1000	200	200

Table 7. Summary of INAA results for NBS-1633a using long- and medium-lived isotope schemes. Values are reported as ppm except * (%).

Element	Radioisotope measured	γ-ray energy counted (keV)	SLOWPOKE result	Certified value
Fe*	^{59}Fe	1099	8.56 ± 0.25	9.40 ± 0.10
Ba*	^{131}Ba	496	0.149 ± 0.004	0.150
K*	^{42}K	1525	2.04 ± 0.05	1.88 ± 0.06
Ni	^{58}Co	810	208 ± 35	127 ± 4
La	^{140}La	487	23 ± 8	-
As	^{76}As	559	151 ± 6	145 ± 15
Br	^{80}Br	554	0.3 ± 0.1	-
Lu	^{177}Lu	208	1.15 ± 0.07	-
Nd	^{147}Nd	531	9 ± 1	-
Mo	^{99}Mo	141	35 ± 0.5	29
Hf	^{181}Hf	482	3.1 ± 0.2	7.6
Sc	^{46}Sc	889	36.1 ± 0.2	40
Cr	^{51}Cr	320	195 ± 4	196 ± 6
Eu	^{152}Eu	1408	3.5 ± 0.2	4
Zn	^{65}Zn	1115	303 ± 12	220 ± 10
Rb	^{86}Rb	1077	128 ± 6	131 ± 2
Co	^{60}Co	1173	40.3 ± 0.9	46
Ta	^{182}Ta	1222	2.1 ± 0.2	-
Sb	^{122}Sb	564	7.02 ± 0.09	7
Th	^{233}Pa	312	25.4 ± 0.2	24.7 ± 0.3
Sm	^{153}Sm	103	17.4 ± 0.2	-
Ce	^{141}Ce	293	150 ± 50	180

Table 8. Summary of INAA results for CCRMP soils SO-2 and SO-4 using short-lived isotope scheme. Results are reported as μg/g except for * (%).

Element	Radioisotope measured	γ-ray energy counted (keV)	SO-2		SO-4	
			SLOWPOKE result	Certified value	SLOWPOKE result	Certified value
Al*	^{28}Al	1779	8.21 ± 0.23 (8.09)†	8.07 ± 0.18	5.89 ± 0.17 (5.73)†	5.46 ± 0.15
Ca*	^{49}Ca	3084	1.83 ± 0.24	1.96 ± 0.10	1.12 ± 0.16	1.11 ± 0.05
Na*	^{24}Na	1368	1.84 ± 0.05	1.90 ± 0.05	1.00 ± 0.03	1.00 ± 0.02
Mg*	^{27}Mg	1014	2.06 ± 0.24 (0.66)†	0.54 ± 0.03	1.62 ± 0.37 (0.67)†	0.56 ± 0.04
V	^{52}V	1434	64 ± 7	64 ± 10	93 ± 7	90 ± 11
Cu	^{66}Cu	1039	-	7 ± 1	-	22 ± 1
I	^{121}I	443	8 ± 5	-	-	-
Ti*	^{51}Ti	320	0.83 ± 0.14	0.86 ± 0.02	0.317 ± 0.067	0.34 ± 0.02
Mn	^{56}Mn	1811	733 ± 35	720 ± 20	606 ± 36	600 ± 20

† Values in brackets have been corrected for interferences from Si and P for Al, and from Al and Si for Mg (see Section 3.2).

library even though the nature of these soils is expected to be different from the sugar cane soils being studied in this work.

The results in Table 8 show good agreement between the certified values and those obtained by the SLOWPOKE procedures employed here. Valid results can thus be expected to be obtained in future analyses of soil samples for Mn, V, Na, Ti, and Ca in a relatively short time. The other elements determinable by this procedure with reasonable accuracy are Co, Eu, Sc, Mo, Ba, As, Cr, Rb, Sb, Th, and Ce (Table 7) of which Co and Mo are of biological importance and As and Cr are normally considered toxic to biological systems. If the total time taken for analysis is not a problem, then the long irradiation, long cool, long count technique can be used for future work.

Even though Al and Mg are not considered essential trace elements, they do influence plant health. Mg is a component of chlorophyll and therefore directly involved in photosynthesis. Excess Al in soils with a pH below 5 is toxic. It interferes with cell division in plant roots and also with uptake, transport and use of elements like Ca, Mg and P [59]. The Al values found in SO-2 and SO-4 are considered higher than the certified values. This is due to interference from Si through the $^{28}Si(n,p)^{28}Al$

reaction and from P through the $^{31}P(n,\alpha)^{28}Al$ reaction. The corresponding F values of 207 and 690, combined with the Si and P concentrations present in the soil, make the n,p reaction of ^{28}Si the major contributor to the high value reported for Al. In SO-2, this reaction contributes 1207 µg/g of ^{28}Al while the ^{31}P reaction contributes only 4 µg/g. When the 1211 µg/g contribution is subtracted from the reported value, a value of 8.09% is obtained, which falls within the 95% confidence limits of the certified value (Table 8). Similarly the Mg values found in SO-2 and SO-4 are considered higher than the certified values due to interferences from Al through the $^{27}Al(n,p)^{27}Mg$ reaction and from Si through the $^{30}Si(n,\alpha)^{27}Mg$ reaction. The corresponding F values of 5.8 and 4070, combined with the Al and Si concentrations present in the soil, make the n,p reaction of ^{27}Al the major contributor to the error in the reported value for Mg. It contributes 13,914 µg/g of ^{27}Mg while the ^{30}Si n,γ reaction contributes only 61 µg/g. The corrected value of 0.66% in Table 8 is obtained when the two contributions are removed from the SLOWPOKE result for SO-2. The short-lived isotope scheme is therefore also valid for Al and Mg provided the concentrations and interference factors of the interfering elements can be determined and corrected for.

Spectral interference has been shown to play a major role in the determination of zinc in soils containing significantly high quantities of Eu and Sc [31]. The 1115 keV line of ^{65}Zn lies between the 1112 keV line of ^{152}Eu and the usually intense 1120 keV line of ^{46}Sc. In this study, both lines contributed significantly to the Zn peak, as indicated by the high result in Table 7. If Zn were to be analyzed by this method, the HP(Ge) detector would have to be replaced by a more discriminating detector system, i.e. one with a higher resolving power.

This technique is also not suitable for the analysis of trace copper. The 1779 keV peak of ^{28}Al is so intense that the background associated with it completely buries the small 1039 keV peak of ^{66}Cu. Since the half-life of ^{28}Al (2.31 min) is smaller than that of ^{66}Cu (5.10 min) [28], a longer decay period would be necessary, but the counts due to other elements would suffer if this alternative were adopted. Thus a separate irradiation and counting would be required.

When the schemes discussed above were used to analyze the samples selected for preliminary study, the results shown in Tables 9 and 10 were obtained. Iron is present in quantities far higher than the trace levels required for plant health, and therefore is not of prime importance in the sampling study. Mn too, is present in large

Table 9. Minor and major element concentrations in soil samples selected for preliminary study by INAA. Results are based on a 350 mg test portion and reported as %.

Element	201-03	201-32	201-48	202-07	202-17	202-48
1. Fe** [±0.27][1]	9.05	9.79	8.95	10.58	8.99	9.10
2. Al* [±0.24]	7.67	7.83	7.55	6.86	6.67	6.73
3. Mg* [±0.49]	1.99	2.00	1.91	1.75	1.55	1.45
4. Ti [±0.12]	1.11	1.15	0.97	1.03	1.00	1.18
5. Ca [±0.19]	1.19	1.11	0.82	0.49	0.80	0.68
6. K [±0.042]	0.842	0.760	0.718	3.046	0.987	1.270
7. Na [±0.037]	0.914	0.831	0.688	0.494	0.615	0.673
8. Mn** [±0.0069]	0.1366	0.1359	0.1157	0.0998	0.0855	0.1111

[1] Values in brackets refer to the relative uncertainty (±1.96 σ) in counting statistics, reported in the same units.

* Not corrected for nuclear interference because quantities of interferent elements are unknown.

** Essential to sugarcane growth in trace amounts.

Table 10. Trace element concentrations in soil samples selected for preliminary study by INAA. Results are based on a 350 mg test portion and reported as µg/g.

Element	201-03	201-32	201-48	202-07	202-17	202-39
1. Sm [±0.1][1]	5.0	5.9	15.6	6.0	6.0	6.5
2. As [±0.7]	1.9	4.1	0.0	3.1	2.4	2.1
3. Hf [±0.1]	5.5	7.4	5.5	5.8	7.2	7.4
4. Eu [±0.08]	1.78	2.35	1.66	1.74	1.89	2.10
5. Cs [±0.2]	0.7	0.0	1.0	1.1	1.1	0.8
6. Ta [±0.1]	0.8	0.8	0.8	0.8	0.9	1.2
7. Th [±0.07]	3.29	0.45	0.38	3.80	4.56	3.88
8. I [±8]	25	28	27	25	25	8
9. Cr [±8]	418	438	689	686	499	407
10. V* [±16]	341	318	285	358	288	283
11. Ba [±33]	328	514	369	235	286	333
12. Ni [±27]	168	174	203	190	148	159
13. Rb [±4]	36	43	43	41	45	44
14. Co* [±1]	47	50	43	33	31	35
15. Sc [±0.1]	21.0	25.4	20.9	19.7	20.5	21.7
16. La [±0.8]	17.2	20.8	19.4	21.3	22.6	22.4
17. Nd [±7]	13	37	28	20	24	21
18. Mo* [±0.4]	12.3	5.6	12.7	13.3	17.5	17.5

[1] Values in brackets refer to the relative uncertainty (±1.96 σ) in counting statistics, reported in units of µg/g.

* Biologically active in trace amounts, especially in enzymatic reactions.

quantities, and so is not an important element to study. The availability of these elements to plants is another matter, of course, as has been discussed earlier for iron. To determine bioavailability requires more information on speciation.

While the distributions of Mn, Fe, Zn, and Cu could be looked at in this study, only zinc and copper were considered because of their observed deficiency symptoms in the sugarcane plants.

4. Conclusions

The INAA technique is very useful for preliminary studies of the type considered here. The results for Mn, V, Cr, Na, Ti, Ca, Cr, Rb, Th, Sb, Ce, As, Fe, Mo, Sc, Co, Eu, Al, and Mg can be assumed to be valid when the accuracy obtained for the standard reference materials is considered. Errors associated with primary nuclear interferences can be significant for Al and Mg, but corrections reduce these errors significantly. Successful application of the corrections depends in part on the uncertainty associated with the interference factors and the fact that the values of F differ somewhat from one nuclear reactor facility to the next.

Because of the high Mn and Fe levels found in the sugarcane soil, INAA would be optimal only for preliminary work on these elements. Atomic absorption spectroscopy (AAS) would most likely be the method of choice in a sampling study because it can yield reliable and relatively rapid results for Cu and Zn, two essential trace elements. Also AAS is conveniently available in our laboratories and is less costly than INAA if only one or two elements are to be determined at the ppm level. Finally, the lack of reactor accessibility in Swaziland makes the use of AAS more attractive.

CHAPTER IV

SAMPLE DISSOLUTION STUDIES FOR ATOMIC ABSORPTION SPECTROSCOPY

1. Introduction

Since zinc and copper could not be easily determined in the soils studied here by instrumental neutron activation analysis, flame atomic absorption spectroscopy (AAS) was chosen as a complementary technique. However, a sample dissolution step, which may be time consuming and hazardous when performed in the conventional way, must be completed prior to instrumental measurement by AAS.

In this section, closed-vessel microwave digestion of soil is compared with conventional open-vessel methods. The effect of time, microwave power and acid composition is also investigated. The technique of standard additions is employed to minimize matrix effects during measurement.

2. Experimental

2.1 Mixing and Particle Size Reduction

All samples were ground to a powder using a Retsch motor-driven agate mortar and pestle with a 0-60 min

variable timer (Brinkmann Instruments, Rexdale, Ontario, Canada). Due to the high clay content of the soil, two stages were employed during the grinding process. The initial stage involved grinding for 3 minutes, followed by scratching of caked clay material along the edges of the mortar. The final 2 minute grinding stage was followed by placing the samples in polyethylene bottles and mixing by tumbling end-over-end for 2 h. To avoid cross-contamination, a few grams of pure silica sand was ground between samples, followed by thorough rinsing of the mortar and pestle with distilled water.

2.2 Procedure for Obtaining Zinc and Copper Calibration Curves

All blanks and standard solutions were prepared using deionized water obtained by passing distilled water through a column of mixed strong acid and strong base ion exchange resins (Amberlite MB-3, Rohm and Haas Co.). All volumetric ware was soaked in 3:1 v/v 68% HNO_3 (analytical reagent, BDH Chemicals, Toronto, Ontario) for at least 30 min prior to use.

A 997 ppm Zn^{2+} stock solution was prepared by dissolving 498.52 mg of zinc metal dust (Fisher Scientific Company, New Jersey, USA) in a minimal amount of 1:1 v/v HCl (analytical reagent, BDH Chemicals), followed by

transfer to a 500-mL volumetric flask and dilution to volume. In a similar manner, a 999 ppm Cu^{2+} stock solution was prepared by dissolving 499.38 mg of sand paper-cleaned, electrolytic copper wire (99.99% pure) in 1:1 v/v 65% HNO_3 (Seastar Chemicals, B.C., Canada; produced by double sub-boiling distillation in quartz) and diluting to 500 mL [60].

Working standards were prepared by pipetting appropriate volumes of the stock solutions using a Socorex micropipette (Terochem Laboratories Ltd., Edmonton, Canada) and diluting to 100 mL. All solutions were subsequently stored in polyethylene bottles (Nalgene Labware).

Atomic absorption measurements were performed on a microprocessor-controlled atomic absorption spectrophotometer (Spectr AA-10, 1985 model by Varian Techtron, Varian Associates, Australia). An in-built deuterium lamp was used for background correction. A Microsoft software package (Varian Techtron, Pty, Ltd., 1985) was used for optimization of instrumental parameters, statistical analysis, data storage, and result generation. All measurements were done in flame mode using air as an oxidant and acetylene as fuel. The overall instrument parameters are listed in Table 11.

Table 11. Instrument parameters for flame AAS measurements.

Parameter	Copper	Zinc
Oxidant flow rate (cm^3/min)	3.0	3.0
Fuel flow rate (cm^3/min	1.5	1.5
Burner vertical height (mm)	8	8
Background correction	"OFF"	"ON"
Wavelength (nm)	324.8	213.9
Spectral band pass (nm)	0.5	1.0
Lamp current (mA)	5	4
Signal delay time (s)	5	5
Signal integration time (s)	5	5
Number of replicates	5	5

2.3 Open-vessel Dissolution of Soil Using HNO_3, HF and $HClO_4$

70% $HClO_4$ (analytical reagent, Mallinckrodt Chemical Works, St. Louis, MO, USA), 49% HF (reagent ACS grade, Fisher Scientific) and purified 65% HNO_3 (Seastar Chemicals) were used for dissolution procedures. CCRMP reference soil standards, SO-1 (partially weathered Champlain sea clay of the C-horizon) and SO-4 (A-horizon of a black chernozemic soil developed in silty glacial lacustrine deposits) were used for validation of the analytical methodologies.

30-mL thick-walled Parr Teflon acid bombs (Parr Instrument Co.) and 50-mL Teflon beakers were used as digestion vessels. All fuming procedures were carried out in a steel fume hood using a sand bath at 200°C. A blank, prepared by running the acid mixtures without the sample through all the procedures, was included.

To a 100-mg dry test portion in a Teflon bomb, 3-mL HNO_3, 2-mL HF and 1-mL $HClO_4$ were added and the bombs were placed in a boiling water bath for 2.5 h. The contents were quantitatively transferred to a Teflon beaker and fumed to near dryness. The resulting residue was dissolved in 1-mL HNO_3 and 1-mL $HClO_4$ and fumed again to near dryness. The colorless residue remaining at this stage was re-dissolved in 2-mL HNO_3 to give a clear solution which was diluted to 50 mL.

To a 500-mg sample in a Teflon bomb, 6-mL HNO_3, 3-mL HF and 2-mL $HClO_4$ were added and the bombs heated at 100°C for 2.5 h. After the fuming procedure 2-mL HNO_3 and 2-mL $HClO_4$ were added to dissolve the residue. The final residue was re-dissolved with 2-mL HNO_3 before diluting to 100 mL. Both procedures took, on average, 10 to 12 h to complete.

2.4 Closed-vessel Microwave Leaching Using HNO_3

The microwave oven used in this study was a 700 W Sears Kenmore (Model 87751) operating at a frequency of 24.5 MHz. It has a variable timing cycle ranging from 1 s to 100 min in 1 s increments, and a variable heating cycle based on power settings ranging from 0% (0 W) to 100% (700 W) in 1% increments. It has a sample handling capacity of eight 60-mL digestion vessels. These screw-cap, wide-mouth vessels were made of Teflon-PFA with a fully fluorinated alkoxy side chain (Savillex Corp., Minnetonka, MN, USA). A Litton Ware microwave transparent rotating platform, purchased at a local drug store was added as an accessory to avoid "hot spots" in certain locations due to inhomogeneities in microwave energy inside the cavity.

Approximately 100-mg samples were weighed into 60-mL Savillex Teflon digestion vessels, 2-mL HNO_3 were added and the lids tightened with the plastic wrench provided. The vessels were placed inside the microwave oven. An initial heating stage of 25% power for 9 min was followed

by a second heating stage of 1% power for 25 min. Cooling at 0°C for 40 min followed, after which the contents were transferred into 50-mL volumetric flasks and diluted to volume. A blank containing all the reagents, but no sample, was also run with each set of samples.

2.5 Closed-vessel Microwave Acid Digestion Using HNO_3 and HF

A maximum of 500-mg portions of soil were weighed into 60-mL Savillex digestion containers. Four mL of 68% HNO_3 and 7 mL of 49% HF were added to each sample. The container lids were tightened with the wrench provided and the set of vessels placed in a ring in a 4-L polyethylene ice cream container with a tight fitting lid. A beaker containing 40 mL of water was also placed inside the oven. The power and time settings were adjusted as indicated in the Results and Discussion section. After digestion and cooling to room temperature, the bombs were opened, the contents transferred to 100-mL volumetric flasks and diluted to 100 mL with deionized water.

2.6 Standard Additions and Atomic Absorption Measurements

For each sample, four 20-mL aliquots of the resultant 100-mL solutions were pipetted into four 50-mL polyethylene volumetric flasks. A Socorex micropipet was used to quantitatively transfer spikes from the stock solutions

of Zn^{2+} and Cu^{2+} into the flasks. Spike volumes ranged from 5 to 75 µL. Deionized water was used to dilute all solutions to volume, after which they were stored in 60-mL polyethylene bottles prior to instrumental measurement.

Spiked samples were aspirated in the standard additions mode of the Varian AA-10 at 324.8 and 213.9 nm wavelengths for Cu^{2+} and Zn^{2+} respectively. The instrumental parameters used are listed in Table 11. Copies of the standard addition plots, together with calculated values for the concentration axis intercepts using linear regression, were obtained from an on-line Epson (Spectrum LX-80) printer. All of these calculations were performed by programs built into the computer software of the AA-10 instrument.

3. Results and Discussion

3.1 Particle Size Distribution

Table 12 shows a typical distribution for the machine ground samples. The weight and cumulative percentage columns are based on sieve weights taken before and after approximately 2 grams of sample were mechanically shaken for 10 min.

The variance due to subsampling, s^2_{ss}, in equation (1) is reduced by particle size reduction and homogenization

Table 12. Particle size distribution for sample number 201-22.

Sieve size (μm)	Weight %	Cumulative percentage
>250	16.31	100.00
180	11.59	83.69
150	15.45	72.10
106	14.16	56.65
90	12.45	42.49
75	7.30	30.04
45	14.59	22.74
<45	8.15	8.15

of the samples. Segregation of larger particles from smaller ones is effectively minimized when laboratory test portions are taken from samples immediately after thorough mixing.

About 75% of the sample measured here consists of particles less than 150 μm and only 15% of the particles are 1/4 mm or greater in diameter (assuming a spherical shape). Although sampling variance is controlled primarily by the largest particles, several portions of the same sample are not expected to differ significantly in Zn and Cu contents on the basis of the observed distribution, partly because much of the zinc is expected to be present as powdered $ZnCl_2$, and partly because both metals are expected to be leached from the particles to a significant extent by the acids employed.

3.2 Zinc and Copper Calibration Curves

Table 13 shows the results obtained when standard solutions of Cu^{2+} were aspirated using the conditions shown in Table 11. The calibration plot is presented in Figure 6. All replicate readings on individual solutions showed a coefficient of variation (% rsd) of 1.5% or less, which indicates adequate reproducibility. Also, blank readings never exceeded ± 0.001 absorbance units for either zinc or copper during the entire period of instrument use.

Table 13. Average absorbance values for 0 to 10 ppm solutions of copper.

Standard	Absorbance (Av. of 5 measurements)	Absolute standard deviation	Relative standard deviation (%)
0.00	0.000	-	-
2.00	0.144	0.001	1.0
4.00	0.283	0.001	0.5
6.00	0.424	0.002	0.4
8.00	0.553	0.008	1.5
10.00	0.682	0.004	0.6

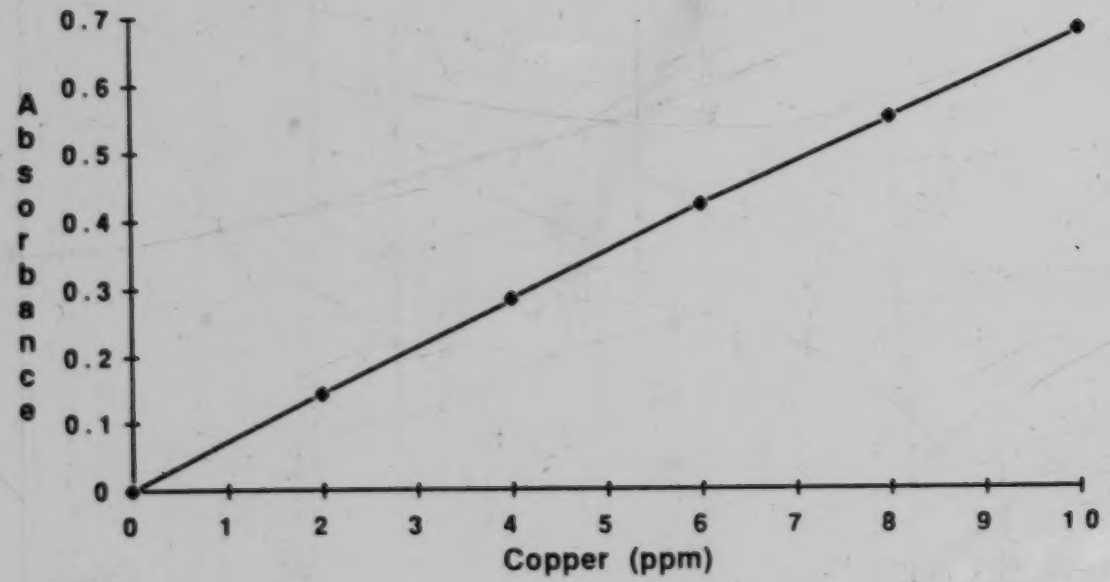

Figure 6. Calibration curve for Cu^{2+} over the range 0 to 6 ppm, r = 0.9997.

The plots obtained were similar to Zn^{2+} and Cu^{2+} calibration plots found in the instrument manual [42]. Beer's Law is obeyed at low concentrations, i.e. the relationship between absorbance and concentration is linear, with a calculated correlation coefficient, r, of 0.9997 for copper in the concentration range covering 0 to 6 ppm. Negative deviations from Beer's Law become apparent from about 7 ppm to higher concentrations, probably due to self-absorption. Table 14 shows data for a zinc calibration; the corresponding plot is shown in Figure 7.

Apart from the 0.100 ppm standard, all solutions showed coefficient of variations of 1.5% or less as in the Cu^{2+} calibration. On the basis of the absolute standard deviation however, the absorbance readings of the 0.100 ppm Zn^{2+} standard are precise.

A calibration plot for zinc over the range 0 to 2 ppm is shown in Figure 8. Two differences between Zn^{2+} and Cu^{2+} are evident. First, the method is approximately 4 times more sensitive for Zn^{2+} than it is for Cu^{2+}. Secondly, negative deviations from Beer's Law appear at much lower concentrations of Zn^{2+}. The 0 to 0.5 ppm range of the calibration plot, in expanded form, is presented in Figure 8, from which it can be concluded that Zn^{2+} obeys Beer's Law at low concentrations, that is, the observed

Table 14. Average absorbance values for 0 to 2 ppm solutions of zinc.

Standard	Absorbance (Av. of 5 measurements)	Absolute standard deviation	Relative standard deviation
0.000	0.000	-	-
0.100	0.046	0.000874	1.900
0.150	0.068	0.001020	1.500
0.200	0.093	0.000837	0.900
0.250	0.111	0.000777	0.700
0.300	0.135	0.000810	0.600
0.500	0.219	0.000657	0.300
1.000	0.358	0.001432	0.400
2.00	0.535	0.001605	0.300

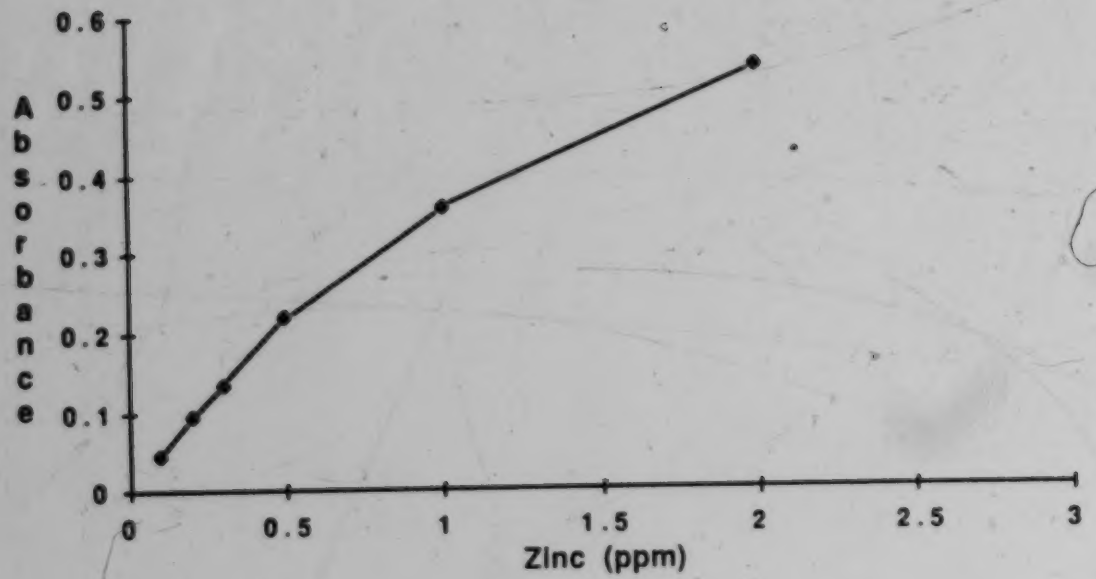

Figure 7. Calibration curve for Zn^{2+}. Curvature begins at about 0.500 ppm.

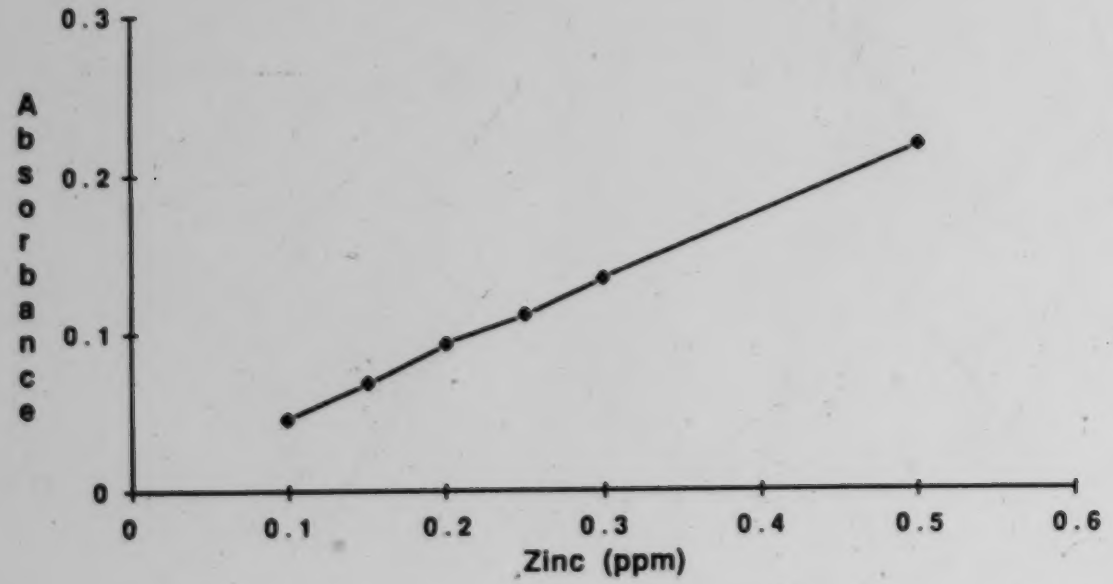

Figure 8. Expanded linear region for Zn^{2+} curve.

positive correlation between absorbance and concentration ($r = 0.9996$) is highly significant. Accordingly, all succeeding measurements of zinc were made on solutions in this concentration range.

3.3 Preliminary Estimates of Zn, Cu and Pb in Soil

Preliminary semi-quantitative atomic absorption measurements were carried out to estimate the quantities of Zn, Cu and Pb in sugarcane soil. According to a study of Pb contamination in road-side grass arising from PbO_2 in automobile exhaust in Swaziland [61], lead concentration decreases with increasing distance from highways.

Open-vessel dissolution (Section 2.3) and microwave leaching (Section 2.4) procedures were carried out on four 100-mg test portions of sample 201-22 which, according to Figure 2, would be expected to exhibit a higher Pb content than samples taken in the middle of the field. The validity of the procedures was evaluated by dissolving 100-mg portions of CCRMP SO-1 by each of the methods. Zn and Cu were determined by flame atomic absorption spectroscopy and quantified graphically by extrapolation of sample absorbances on calibration plots which were run on the same day. Lead was determined by graphite furnace atomic absorption spectroscopy [62] and quantified by

standard additions. For the purpose of comparing microwave leaching and perchloric acid dissolution, alternation between leached and completely dissolved sampls was employed. Evaluation of instrument drift was done through the use of blanks between sample runs. Table 15 provides a summary of the results obtained in this study.

The results obtained for SO-1 by total $HClO_4$ dissolution are significantly higher than the certified values. Cu is about 7 ppm above the tolerance limit of 64 ppm while Zn is higher by 6 ppm (on the assumption that the value of 209 is an outlier, which was confirmed by subsequent measurements). The problem of matrix effects is more likely to contribute to the observed systematic error. The matrix seems to enhance the Zn and Cu signals in the sample. Unless standards and samples are identical in matrix composition, results obtained in future analyses are likely to be biased high. However, for purposes of this preliminary work, it can be concluded that both elements are present in readily measurable trace quantities in the soil and their distribution in the field is worth investigating.

The GFAAS results for Pb also indicate the presence of this metal at trace levels in the soil. It was decided that it would be counterproductive cost wise to pursue

Table 15. Comparison of total dissolution with HNO_3 leaching for copper, zinc and lead determinations on 100-mg portions of soil sample 201-22. All values are in μg/g. Copper and zinc values read directly from calibration curves, lead by standard additions.

Subsample	Zinc		Copper		Zinc
	total[1]	leached[2]	total	leached	total
A	94	47	139	91	12
B	90	46	140	90	7
C	95	50	135	87	9
D	95	44	133	82	12
SO-1[3]	157	136	71	54	17
SO-1	209	132	71	51	

[1]Refers to dissolution procedures outlined in Section 2.3.

[2]Refers to digestion procedures outlined in Section 2.4.

[3]Certified values for SO-1 are 146 ± 5 for Zn, 61 ±3 for Cu and 22 ± 4 for Pb.

further studies on Pb distribution in the field at this time, although analysis of sugarcane or sugar products for lead might be of interest in the future as a separate project.

The results also show clearly that total dissolution by perchloric acid is superior to leaching with nitric acid in the release of Zn and Cu in this soil. Leaching extracts only about two-thirds of the Cu, and less than one-half of the Zn obtained by total dissolution of the soil sample studied.

3.4 Analytical Measurement Uncertainties

To assess the approximate magnitude of the errors associated with day-to-day variations in instrument performance, i.e. s_a in equation (1), repeat measurements over a 3-day period were carried out on several 500-mg portions of sample 201-22 dissolved by the total dissolution procedure (Section 2.3). A standard additions procedure seemed to be advisable in order to suppress matrix effects and was therefore the method evaluated for subsequent analyses.

Using the approximate Zn and Cu values for sample 201-22 as a guide (Table 15), the procedures described in Sections 2.3 and 2.6 were employed. Here 0, 25, 50, and 75 µL spikes of 999 ppm Cu^{2+} were added to 20-mL aliquots

of sample solution. Similarly, 0, 5, 10, and 15 μL spikes of 997 ppm Zn^{2+} were added. The results for one subsample (201-22-1) are shown in Table 16 and plotted in Figures 9 and 10.

Table 16. Total dissolution and standard addition results for a 500.21 mg test portion of sample 201-22.

Addition	Copper absorbance	Zinc absorbance
0	0.044	0.086
1	0.133	0.130
2	0.221	0.171
3	0.305	0.208

The linear regression intercept, V_C, is given by the equation:

$$V_C = \frac{(\Sigma A)(\Sigma V^2) - [\Sigma(AV)](\Sigma V)}{(n)\ [\Sigma(AV)] - (\Sigma A)(\Sigma V)} \qquad (15)$$

and the variance about this intercept value, s_{VC}, by the equation:

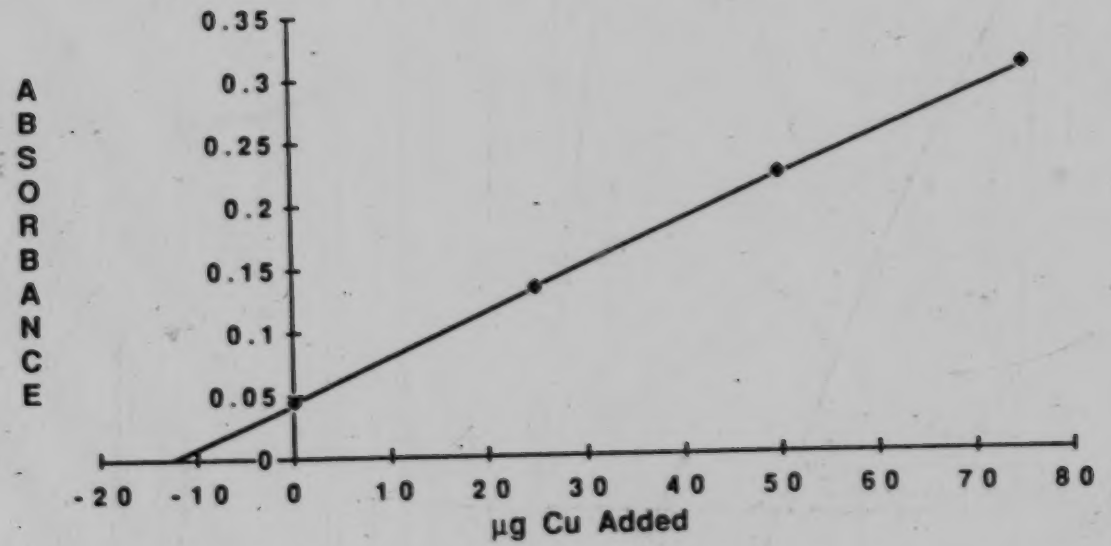

Figure 9. Absorbance *vs* micrograms of spike in sample for Cu. Intercept: -12.4 µg/g, uncertainty: 0.139 µg. Last data point excluded in calculations.

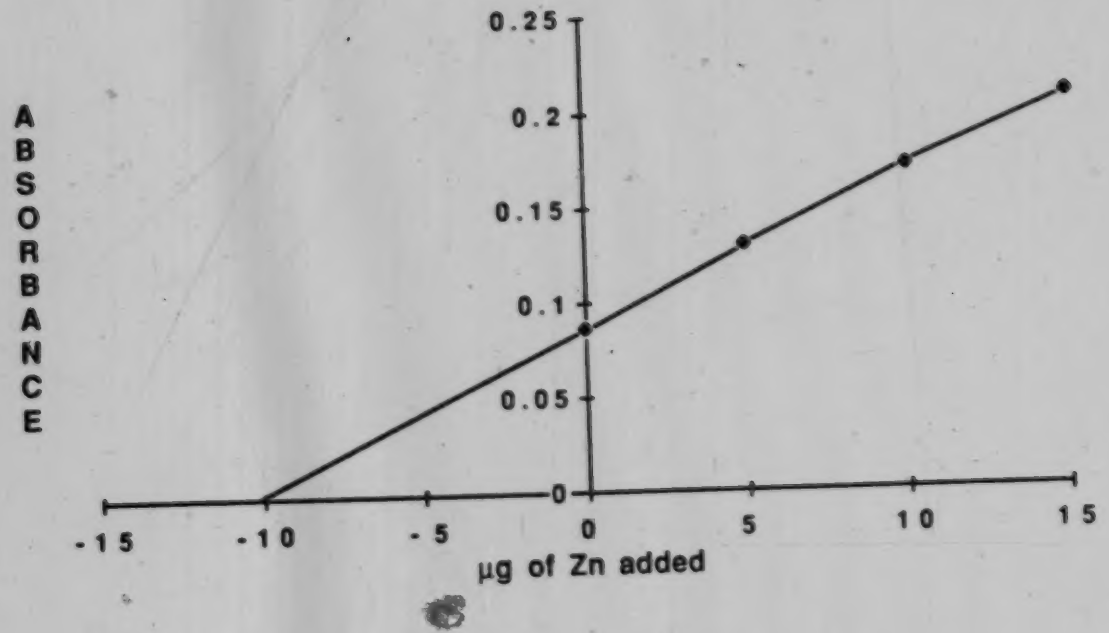

Figure 10. Absorbance *vs* micrograms of Zn^{2+} spike added. Intercept: -10.18 µg, uncertainty: 0.142 µg. Last data point excluded in calculations.

$$s_{Vc}^2 = \frac{\Sigma d^2}{n-2} \cdot \frac{\Sigma(V + V_c)^2}{[\Sigma A_{calc}]^2} / \left[\frac{n\,\Sigma(V + V_c)^2}{[\Sigma(V + V_c)]^2} - 1\right] \qquad (16)$$

In these equations, A is the absorbance, V is the volume of spike added in μL, and A_{calc} is the calculated absorbance, using the relation:

$$A_{calc} = m(V + V_c) \qquad (17)$$

Here m is the slope of the least-squares line obtained from:

$$m = \frac{\Sigma A}{\Sigma V + nV_c} \qquad (18)$$

where n is the number of data points in the plot and d is the difference between A and A_{calc} for each data point [63].

The calculated value of V_c is -12.5 μg and the value of s_{Vc} is 0.139 μg, corresponding to a concentration of 125 ppm and an absolute standard deviation of 1 ppm for Cu. The zinc content is 102 ppm and the absolute standard deviation is 1 ppm. In the calculations, the last data point (addition 3 in Table 16) was omitted because it falls outside the linear range in both cases. A relative standard deviation of 1% is small, therefore the standard

additions and measurement procedures can be considered sufficiently precise for both elements. Similar behavior was observed in the other 3 subsamples studied. The overall results are shown in Table 17.

Since each value reported has an uncertainty s_{vc} associated with it, this error is also included when data taken on different days is evaluated. It is possible to separate this error from the measurement error by equation (1):

$$s^2_{aa+vc} = s^2_{aa} + s^2_{vc}$$

If s^2_{vc} is taken as 1 ppm and s_{aa+vc} as 2 ppm (the average value from Table 17), s_{aa} turns out to be about 2 ppm for both elements. An error of about 2% is therefore expected to be associated with day-to-day variations in instrument performance. However, this value should be taken as a rough estimate since it is based on only a few measurements. To obtain a better estimate of the true measurement/analytical uncertainty, σ_{aa}, s_{aa} would have to be based on a larger number of runs.

3.5 Uncertainties Due to Subsampling

To assess the error associated with subsampling procedures, several 500-mg portions from selected samples

Table 17. Copper and zinc concentrations in 4 test portions of sample 201-22. Samples totally dissolved with $HClO_4$ and run on different days, using a standard additions procedure.

	Copper (ppm)				Zinc (ppm)			
Parameter	A	B	C	D	A	B	C	D
day 1	125	126	125	116	102	102	98	102
day 2	124	127	120	119	100	100	102	106
day 3	126	124	129	116	102	100	100	100
$\bar{x}$	125	126	125	117	101	101	100	103
s_{aa+vc}	1	2	4	2	1	1	2	3

were completely dissolved and analyzed by the standard additions procedure described in Section 3.4. The results obtained are presented in Table 18.

The standard deviations shown include s_{vc}, s_{aa} and the uncertainty associated with variations between test portions taken from the same sample, s_{ss}. According to equation (1),

$$s^2_{vc+ss+aa} = s^2_{vc} + s^2_{aa} + s^2_{ss}$$

Since soil is particulate in nature, it is expected to show a certain degree of heterogeneity which is reflected by the magnitude of s_{ss}. Taking the worst possible case ($s_{vc+aa+ss}$ = 4 ppm) in the samples studied for Cu and Zn, equation (1) yields $\sqrt{11}$ (=3 ppm) as the expected error associated with the subsampling procedure. This is only an estimate, and would improve as the number of subsamples investigated is increased.

3.6 Validity of Analytical Procedures

To evaluate the accuracy of the dissolution (Section 2.6) schemes, a 500-mg portion of CCRMP SO-4 was analyzed. A value of 95 ppm with an s_{vc+aa} value of 2 ppm for Zn was obtained. This value is in excellent agreement with the certified value of 94 ± 3 ppm at the 95%

Table 18. Estimation of subsampling variability in Zn and Cu concentrations in five samples of Swaziland soil digested with $HClO_4$.

Sample	Subsamples A	B	C	D	$\bar{x}$ (ppm)	s (ppm)
	Zinc (ppm)					
201-22	101	101	101	103	101	1
201-32	102	95	99	94	98	4
201-24	92	92	91	92	92	1
201-01	100	106	104		103	3
201-11	104	103	106		104	2
	Copper (ppm)					
201-22	125	126	125	117	123	4
201-32	145	146	144	147	146	2
201-24	129	130	125	129	128	2
201-01	135	139	136		137	2
201-11	130	128	131		130	2

confidence level. Similar conclusions can be drawn for Cu, for which the experimentally found value was 22 ± 2 ppm, compared to the certified value of 22 ± 1 ppm. Even though the total dissolution method is both hazardous and time consuming, it is valid and reliable, and can therefore be considered a referee method for evaluating other procedures.

3.7 Microwave Digestion

The microwave acid digestion procedure described in Section 2.5 was developed to overcome the shortcomings of using HNO_3 alone, i.e. be able to exhibit the validity and reliability of the referee method at shorter dissolution times. This is especially important considering the large number of analyses to be performed in this study.

(a) Effect of acid mixture composition

The procedure described in Section 2.5 does not require the use of $HClO_4$. It uses HF to assist in the breakdown of complex silicates which are resistant to attack by HNO_3. However, great caution needs to be exercised when handling HF because of its corrosive nature; once in contact with the skin, it causes irreversible tissue damage unless flushed away immediately.

The preliminary results presented in Table 19 were obtained from HNO_3 and HF digestion using a scheme which involved an initial 15 min exposure to microwaves at 15% power, followed by cooling and venting of the Teflon vessels. The venting step was necessary to release pressure build-up by acid fumes and gaseous oxidation products formed according to equation (10). Excess pressure was found to cause leakage of the contents and also would present an explosion hazard. After retightening, the vessels were treated for 60 min at 30% microwave power.

It is evident that HF and HNO_3 as a mixture is more efficient at extracting Cu and Zn from the soil matrix than HNO_3 alone. However, only about 90 to 95% of the sample was dissolved. The residue remaining after the microwave digestion consists of the more refractive minerals, which on the basis of results shown in Table 19 do not appear to contain any Zn or Cu.

(b) Effect of time

The results shown in Table 20 were obtained by microwave digestion at longer dissolution times. The reported times do not include the initial 15% power - 15 min venting stage. Venting was necessary after every 30 min for the first 2 h of digestion and every 60 min thereafter. The extent of dissolution was monitored by

Table 19. Zinc and copper in soil samples digested by microwave procedures. Uncertainties given as one standard deviation.

	Sample 201-32		Sample 201-24	
Subsample	Zn (ppm)	Cu (ppm)	Zn (ppm)	Cu (ppm)
A	94	149	92	126
B	102	144	89	130
C	93	142		
D	94	142		
E	91	143		
Average	95±4	144±3	90±2	128±3
Referee method[1]	98±4(4)	146±2(4)	92±1(4)	129±1(3)

[1]Values in brackets refer to the number of subsamples analyzed.

Table 20. Effect of time on microwave digestion of soil samples from Swaziland. Sample 201-32 results are based on one test portion at each time period; sample 201-24 results are based on two test portions.

	Sample 201-32		Sample 201-24		
Time (h)	Zn (ppm)	Cu (ppm)	Zn (ppm)	Cu (ppm)	% Dissolution
1	93	143	90±2	128±3	95
2	95	142	89±1	127±1	94
3	98	143	91±3	127±0	95
4	95	142			92
6	84†	143			90
Referee Method	98±4(4)	146±2(4)	92±1(4)	129±1(3)	

†Error likely due to local heterogeneity since the Cu result on the same portion falls within acceptable limits.

subtracting the weight of dry residue left after digestion from the initial weight of sample.

The results indicate that no advantage is gained by using digestion times longer than 1 h at 30% microwave power, but rather it is disadvantageous to do so because of time constraints and deterioration of the digestion vessels. Even with pressure release after 30 min, there appear to be tremendous pressures developed during the digestion. After these experiments, the vessels bulged permanently around the seals.

(c) Effect of microwave power

Higher power settings were investigated with the aim of finding the optimum setting which would complete the extraction of Zn and Cu from the soil matrix in the shortest possible time while keeping pressure build-up at a minimum. Tables 21 to 23 show results obtained for Cu and Zn at power settings of 35% (300 W), 50% (400 W) and 100% (700 W) respectively.

In Figures 11 and 12, the solid line represents the mean estimates of the population obtained by the referee method and previous microwave digestions. The broken lines are the 95% confidence limits based on the standard deviation estimates. Samples which have been digested adequately should generally fall within these limits; if much below them, probably the extraction was not

Table 21. Microwave digestion of sample 201-24 at 35% power. Data is based on one 500-mg test portion at each time.

Time (min)	Zn (ppm)	Cu (ppm)
5.00	88	121
10.00	91	123
15.00	91	129
20.00	94	128
Referee method	92±1(4)	129±1(3)

Table 22. Values for zinc and copper analyses after microwave digestion of sample 201-24 at 50% power.

Time (min)	Zn (ppm)	Cu (ppm
1.00	84 ± 2 (2)	122 ± 2 (2)
2.00	88	127
3.00	90 ± 2 (2)	128 ± 2 (2)
4.00	91	129
5.00	92 ± 2 (3)	130 ± 1 (3)
7.00	94	128
9.00	94	131

Table 23. Microwave digestion of sample 201-24 at 100% power.

Time (min)	Zn (ppm)	$\bar{x}_{Zn}$ (ppm)	Cu (ppm)	$\bar{x}_{Cu}$ (ppm)
1.00	87		124	
	† 88	88	124	124
2.00	90		127	
	† 89	90	127	127
3.00	82*		124*	
	† 93	93	128	128
5.00	† 91	91	128	128

†65% HNO_3 (BDH Chemicals) used instead of 68% HNO_3 (Seastar) obtained by sub-boiling double distillation in quartz.

*Leakage of bomb contents was observed. These values were not included when calculating the averages.

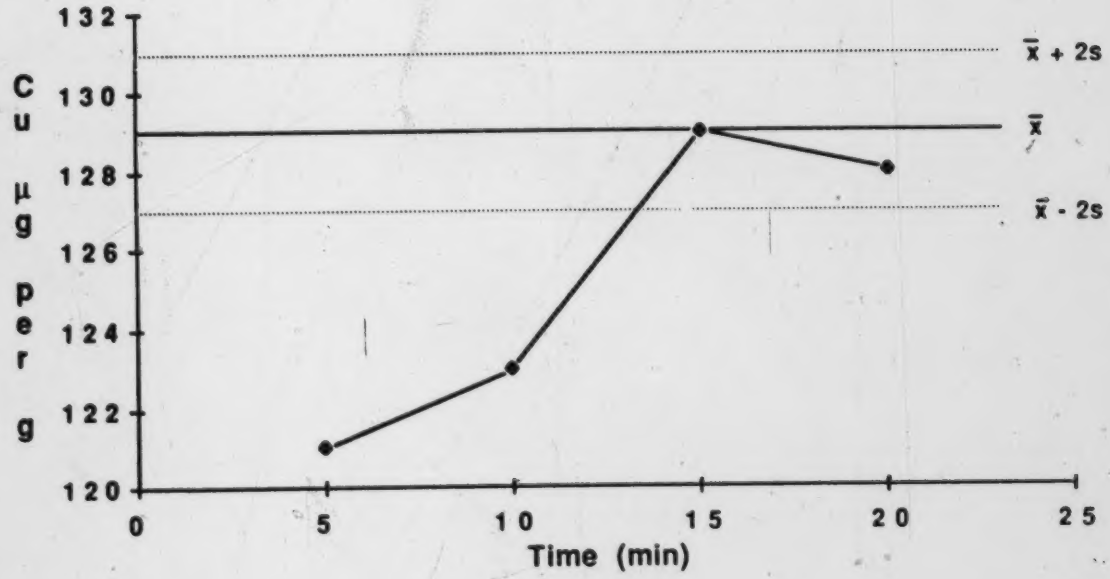

Figure 11. Copper results *vs* time for microwave digestion at 35% power. Sample 201-24. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$

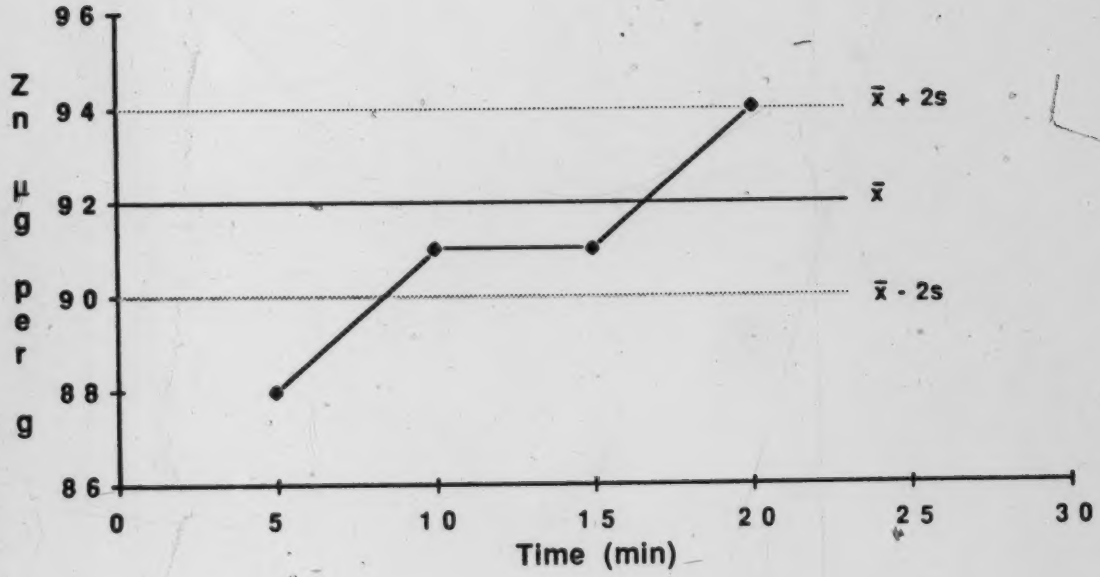

Figure 12. Zinc results *vs* time for microwave digestion at 35% power. Sample 201-24. $\bar{x} + 2s = 196\sigma$, $\bar{x} - 2s = 1.96\sigma$.

complete. This approach is similar to that used in control charts in quality assurance.

From Figures 11 and 12, it is evident that at 35% power, adequate dissolution does not occur until after 15 min for Cu and 10 min for Zn. These figures are only rough estimates because they are based on 1 test portion per data point.

Zinc and copper analyses of sample 201-24 after microwave treatment of several test portions for times ranging from 1 to 9 minutes are summarized in Table 22 for 50% power and 23 for 100% power. For these runs, the corresponding plots are shown in Figures 13 through 16. The corresponding plots indicate that at 50% microwave power, complete extraction of zinc and copper occurs after 4 minutes. The low results shown for copper and zinc in Table 23 were obtained because of leakage of the bomb contents when test portions were exposed to microwaves at full power (700 W) for 3 minutes. Pressure build-up during this time was enough to cause significant deformation of the digestion vessels. When a second run was done with venting after 2 minutes, the results were satisfactory and no leakage or deformation was observed. Acceptable results were also obtained when a test portion was vented after the first 2 minutes and after every 1 1/2 minutes thereafter for 5 minutes. Continuous heating in

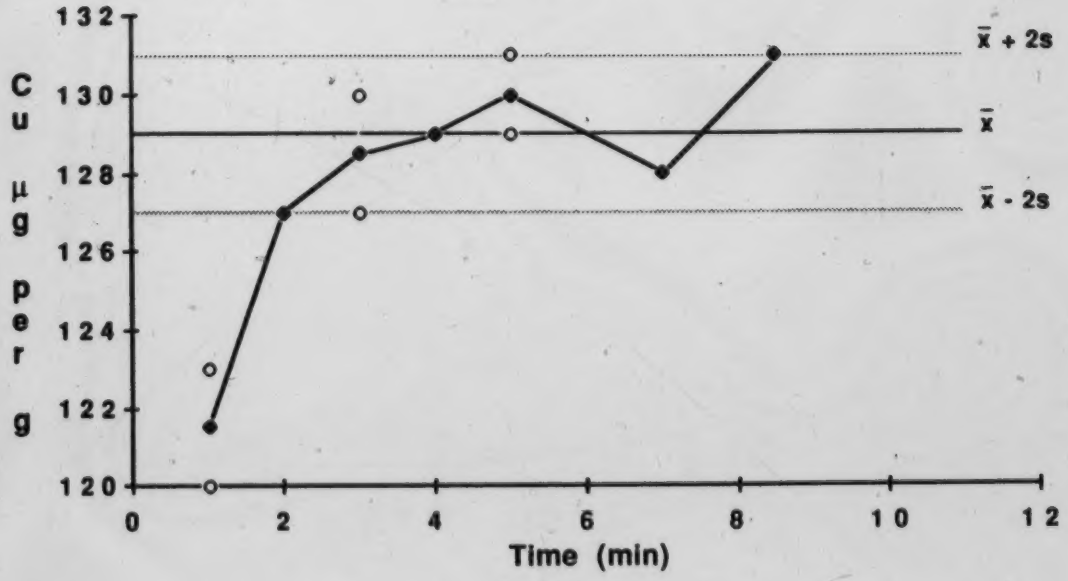

Figure 13. Copper results *vs* time for microwave digestion at 50% power. Sample 201-24. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$. ♦ is the average of values shown by ◊.

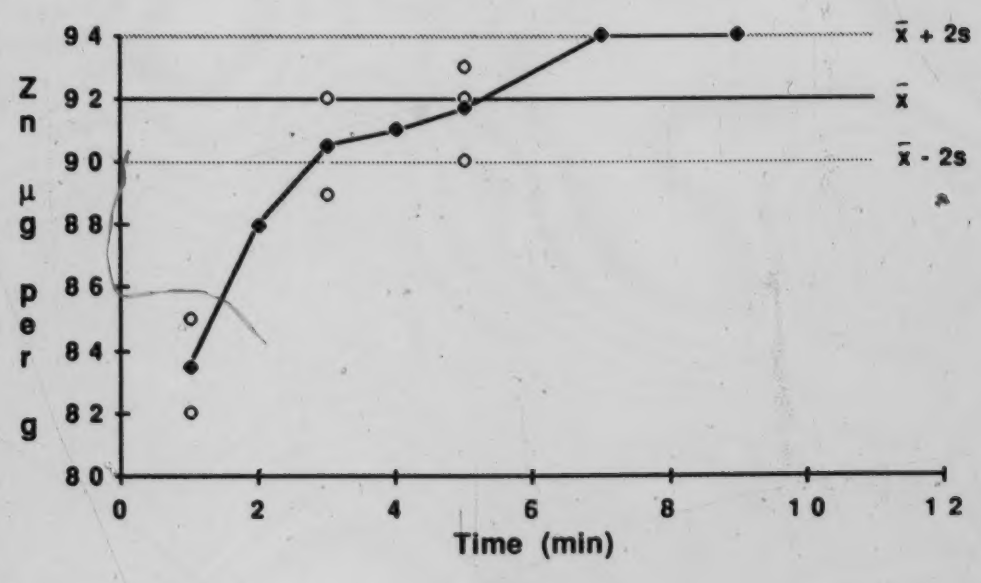

Figure 14. Zinc results *vs* time for microwave digestion at 50% power. Sample 201-24. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$. ♦ is the average of values shown by ◊.

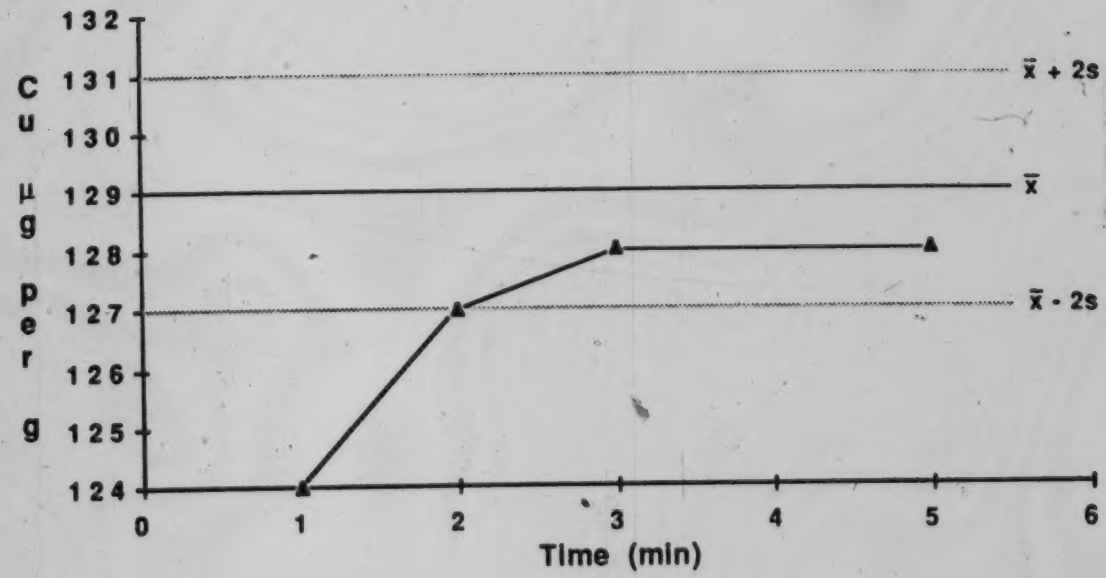

Figure 15. Copper results *vs* time for microwave digestion at 100% power. Sample 201-24. $\bar{x} + 2s = 1.96\ \sigma$, $\bar{x} - 2s = 1.96\sigma$.

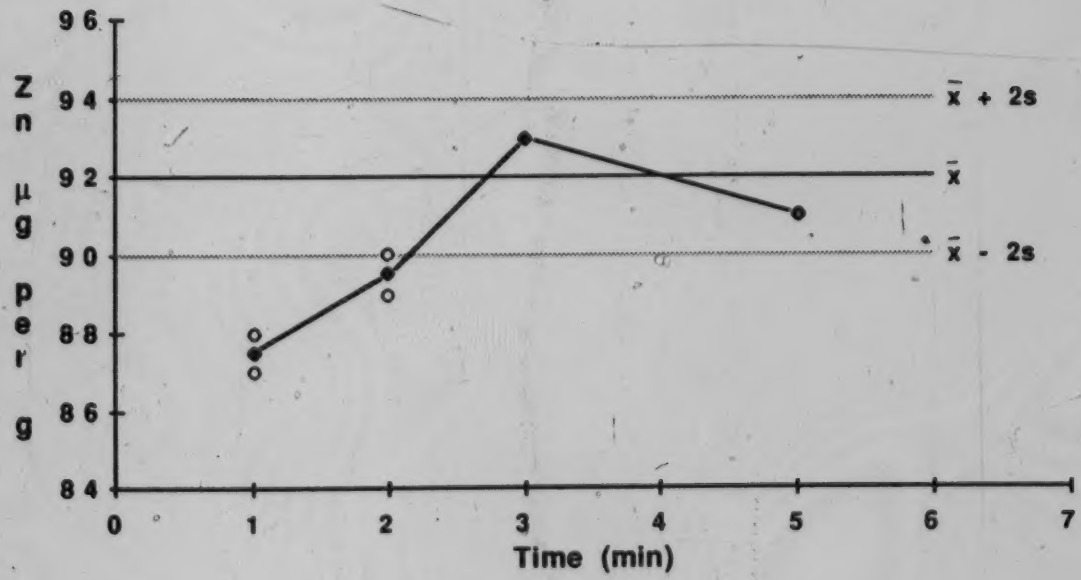

Figure 16. Zinc results *vs* time for microwave digestion at 100% power. Sample 201-24. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$. ♦ is the average values shown by ◊.

excess of 2 minutes is therefore not recommended for this power setting. It can be concluded from these tests that the time required to extract most of the zinc and copper at 100% power is about 2 1/2 minutes.

Figures 17 and 18 show that there is a decrease in the time required to achieve complete extraction of Zn and Cu as microwave power increases. At the same time, however, the risks of sample loss and container deformation or even explosion of the vessels increase. Full power is not recommended for future digestions since there is no significant decrease in dissolution time when compared to 50% power. On the basis of convenience, safety and fast dissolution, 50% was the chosen power setting for future analyses using this method. Even though complete recovery of zinc and copper is observed for the samples investigated after 4 to 5 minutes digestion at this power setting, 7 minutes was considered a safer time to use. This was designed to take care of samples which would prove more difficult to digest than sample 201-24.

Due to the high cost of Seastar specially purified HNO_3, BDH HNO_3 was investigated in its place for use in the digestions. The results show that the difference in purity between these two sources is insignificant for this study. Accordingly, the BDH material was used for all subsequent work.

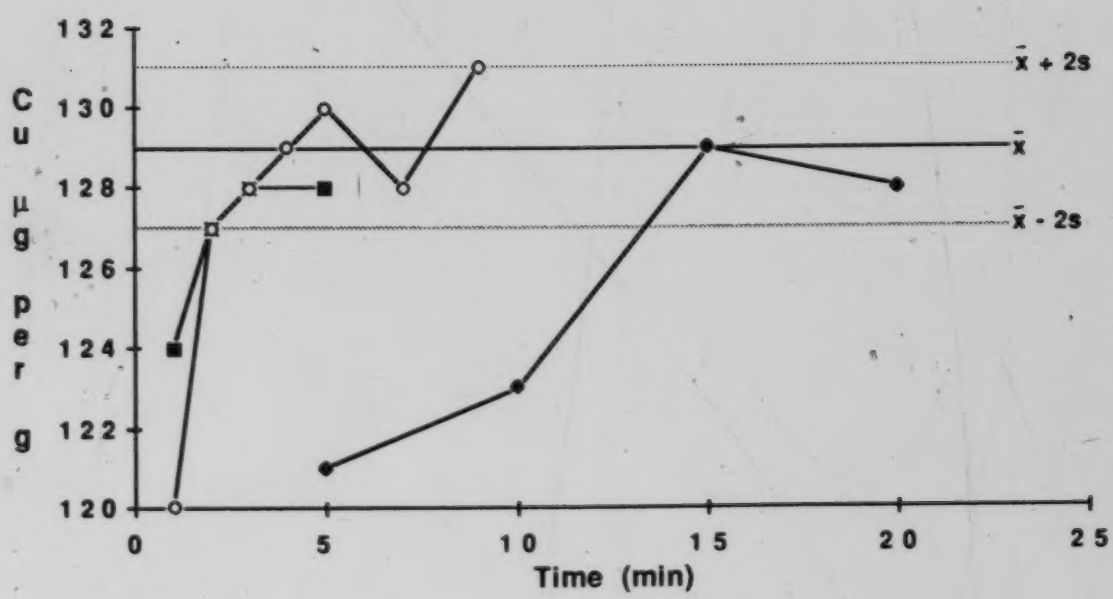

Figure 17. Copper results *vs* time for microwave digestion at 35 (♦), 50 (◊) and 100% (■) power. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$.

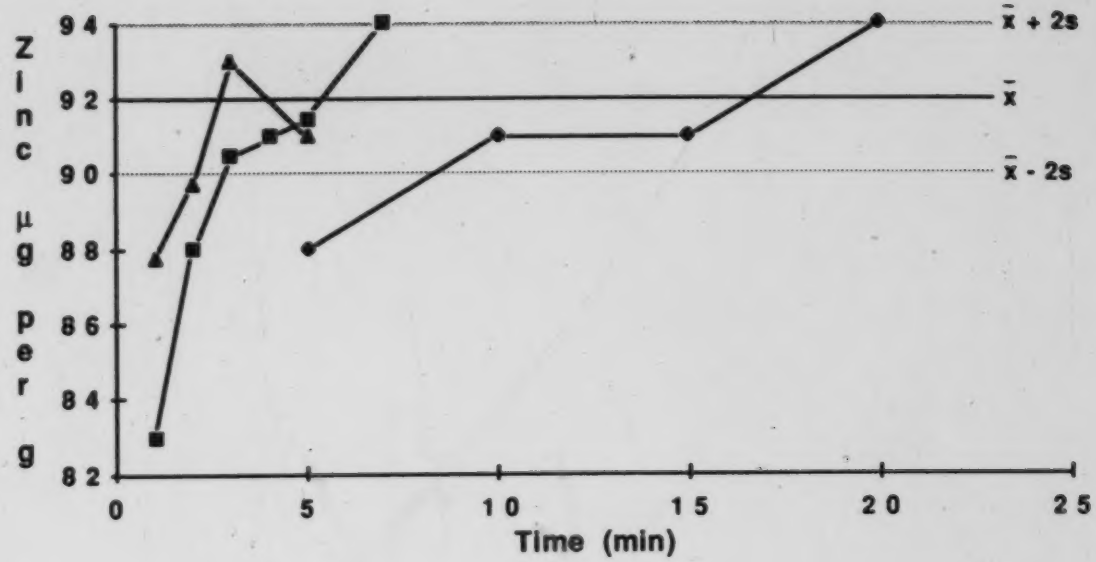

Figure 18. Zinc results *vs* time for microwave digestion at 35 (♦), 50 (■) and 100% (▲) power. $\bar{x} + 2s = 1.96\sigma$, $\bar{x} - 2s = 1.96\sigma$.

(d) Reliability of microwave acid dissolution

Standard reference materials (CCRMP SO-2, SO-3 and SO-4) and several randomly selected samples from field 201 were analyzed using the HF-HNO_3 microwave dissolution scheme at 50% power for 7 min. The results were compared with those obtained by a total dissolution with $HClO_4$, HNO_3 and HF as a mixture. The results are presented in Table 24.

There is no significant difference between the means obtained by the two methods for zinc and copper in the soil samples from Swaziland in Table 24. The results for microwave dissolution show reasonable agreement with certified values for SO-2, SO-3 and SO-4. On this basis, it can be concluded that microwave acid dissolution is reliable, rapid and convenient, and is recommended as a method for preparation of soil test portions for trace element analysis.

4. Conclusions

The microwave acid dissolution procedure described here gives the same results, but is faster and safer than open-vessel methods which employ HF, HNO_3 and $HClO_4$. However, since 100% dissolution of test portions may not be achieved by this method, tests have to be carried out

Table 24. Comparison of referee dissolution method and microwave acid digestion at 50% power for 7 minutes for the determination of zinc and copper in soils.

	Zinc (ppm)		Copper (ppm)	
Sample	Referee method	Microwave digestion	Referee method	Microwave digestion
201-32	98 ± 3	95 ± 2	146 ± 2	144 ± 1
201-22	101 ± 1	100 ± 1	125 ± 1	125 ± 1
201-24	92 ± 1	94	128 ± 2	128
201-11	104 ± 2	101 ± 1	129 ± 1	124 ± 1
201-14	90	88 ± 2	117	118 ± 2
201-06	100	97 ± 1	118	119 ± 1
201-06	103	96 ± 1	137	135 ± 2
201-17	84	83	119	119
201-48	103	97	149	150
SO-2[1]		125		9
SO-3[2]		51		19
SO-4[3]	95	95	25	24

Certified values:

[1]Zn = 124 ± 5; Cu = 7 ± 1

[2]Zn = 52 ± 3; Cu = 17 ± 1

[3]Zn = 94 ± 3; Cu = 22 ± 1

to ensure that the insoluble residue does not contain significant quantities of the element(s) being investigated.

Other soil types besides the one investigated in this study may require different dissolution conditions, and hence the optimum conditions described here (7 min at 400 W power for 8 vessels, each containing a 500-mg test portion of soil) should be used only as a guide. Pressure build-up inside the digestion vessels may cause leakage of contents and possibly explosions if the samples being digested contain large quantities of organic matter which generate high internal pressure upon heating. Therefore caution and close supervision are recommended.

CHAPTER V

EVALUATION OF HETEROGENEITY OF THE SAMPLE SET AND DESIGN OF SAMPLING PLANS FOR TRACE ELEMENT ASSESSMENT IN SOILS

1. Introduction

In this section, the overall heterogeneity of trace zinc and copper in the population, which consists of all the soil in field number 201 to a depth of 10 cm, and covering a total area of 17 ha, is evaluated. A total of 52 soil samples taken from this field were analyzed for zinc and copper. Estimates of the population mean and sampling standard deviation were used in the design of future sampling plans for trace Cu and Zn assessment in the soil of other fields in the ranch.

2. Theory

Several equations for estimation of the individual increment size and number required to achieve a desired precision in soil analyses are presented in Chapter I, Section 2. In that section, it was noted that the equations by Benedetti-Pichler and by Gy cannot be used in

this study. Also, because of problems associated with the evaluation of sampling constants A and B, the Visman equation cannot be applied. This is so because in order to calculate A in equation (7), two sets of increments of widely differing weights, W_{lg} and W_{sm}, are used to obtain sampling standard deviation s_{lg} and s_{sm}. These values are substituted into the equation

$$A = \frac{W_{lg}W_{sm}}{W_{lg} - W_{sm}} (s_{sm}^2 - s_{lg}^2) \qquad (19)$$

to provide a value for the constant A. The value of B is then calculated from

$$B = s_{lg}^2 - \frac{A}{W_{lg}} \qquad (20)$$

Alternatively A can be evaluated by calculating the intraclass correlation coefficient r for increments collected in pairs. A simultaneous solution of equation (20) and the equation

$$r = B/A_m \qquad (21)$$

where m is the reciprocal of the average particle mass of the soil, yields A and B [21]. Upon considering the

procedures which were used to take samples from field number 201 (Chapter II, Section 3.3), it is evident that neither approach can be applied in this study.

The student's t-test equation (8), however, appears more likely to be applicable here. In this equation, prior knowledge of the variance due to sampling, s_s^2, and the average $\bar{x}$, need to be known in order to calculate the minimum number of samples to be taken from the population for a desired level of precision at a given confidence level. In this study, this information was obtained from the analysis of 52 soil samples for copper and zinc. In order to obtain valid estimates of the minimum sample number, n, by this equation, two conditions must be satisfied. First, the sought-for component must show a Gaussian distribution within the population. Secondly, a constant increment size should be used to evaluate the sampling standard deviation s_s since this value may depend greatly on the size of individual samples [9]. Since sample sizes for field 202 are twice those of field 201, the preliminary estimates of population parameters s_s and $\bar{x}$ of field 201 cannot be used to predict n for field number 202.

3. Experimental: Determination of Zinc and Copper in Soil Samples from Field Number 201

A total of 52 soil samples taken from sugarcane field 201 in Swaziland (see Chapter 2, Section 3.3 for details) were analyzed for copper and zinc by atomic absorption spectroscopy. Microwave acid digestion with HF and HNO_3 (Chapter 4, Section 25), followed by standard additions (Chapter 4, Section 2.6) was used to prepare all samples in this study. The results are based on analyses of several 500-mg test portions per sample for sample numbers 201-01 through 201-35 and one 500-mg test portion for numbers 201-36 through 201-52, with occasional replicate analyses in the latter group as a check on the procedures.

Up to eight Savillex Teflon 60-mL vessels were simultaneously heated in a microwave oven at 50% power for 7 minutes. Each set included a reagent blank and a test portion of sample 201-32 which was digested and analyzed with each set to monitor the quality of the analytical procedures. The values for sample 201-32 were plotted on a control chart to ensure that the methodologies were in a state of statistical control.

4. Results and Discussion

4.1 Quality Assurance

The control charts shown in Figures 19 and 20 are sequential plots of zinc and copper results for sample 201-32. Each plot consists of a central solid line at the average value $\bar{x}$, a pair of inner limit lines (warning limits) at $\bar{x} \pm 2s$ and a pair of outer limit lines (action limits) at $\bar{x} \pm 3s$. Values for $\bar{x}$ and s were established from analysis of 23 test portions of sample 201-32 for zinc and copper. The 23 test portions include all results obtained previously from the evaluation of microwave dissolution procedures (see Section 3) and 10 analyses used in quality control. Results of the latter group are shown in Table 25.

The control charts have warning limits at 94 ± 6 and 145 ± 6 ppm for zinc and copper, corresponding to confidence levels of 95%. Since the probability that a particular result falls outside these limits is 0.05 or one in 20, all procedures should be halted and evaluated for possible sources of systematic error if two successive data points fall outside these limits. Similarly, action limits at 94 ± 9 and 145 ± 9 ppm for zinc and copper correspond to a confidence level of 99.8%. Here, the probability level is 0.002 or one case in 500, and action

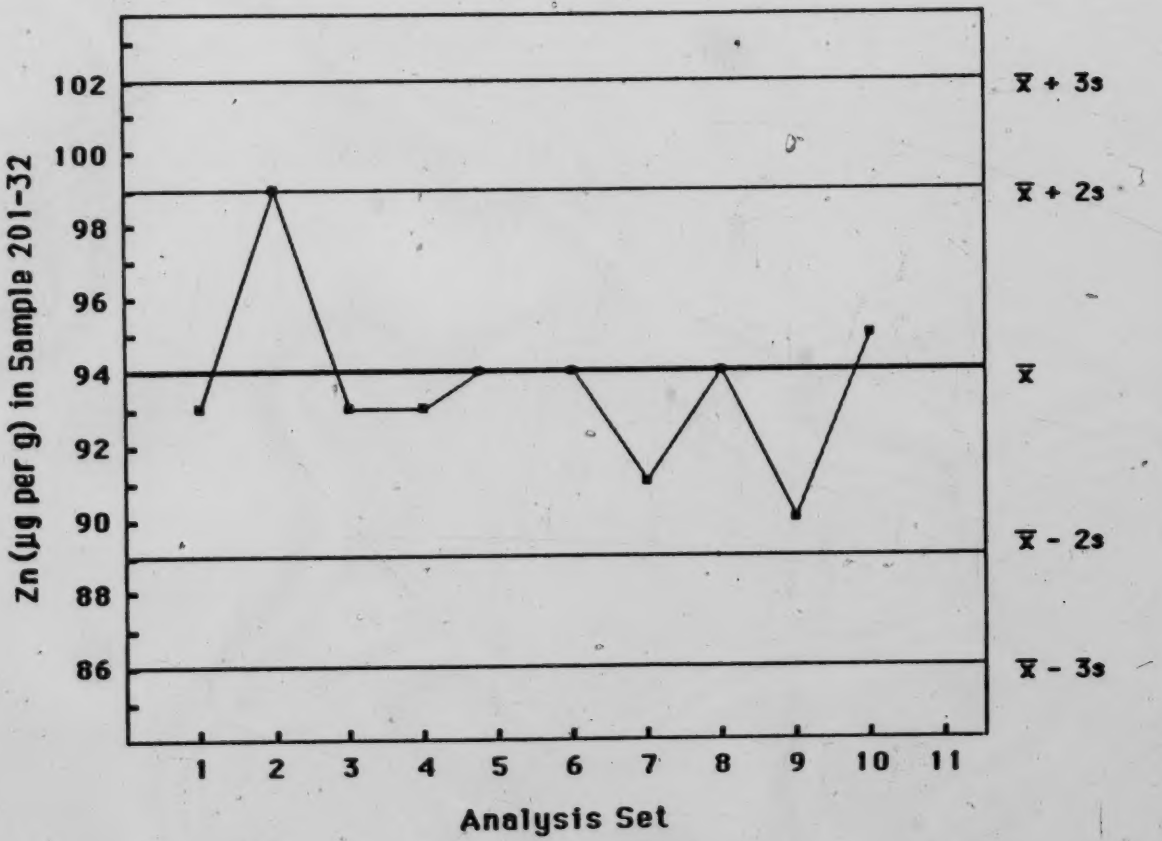

Figure 19. Control chart for zinc analyses of soil samples from field 201. Results for analysis sets were obtained on different days (see section 5.3 for details).

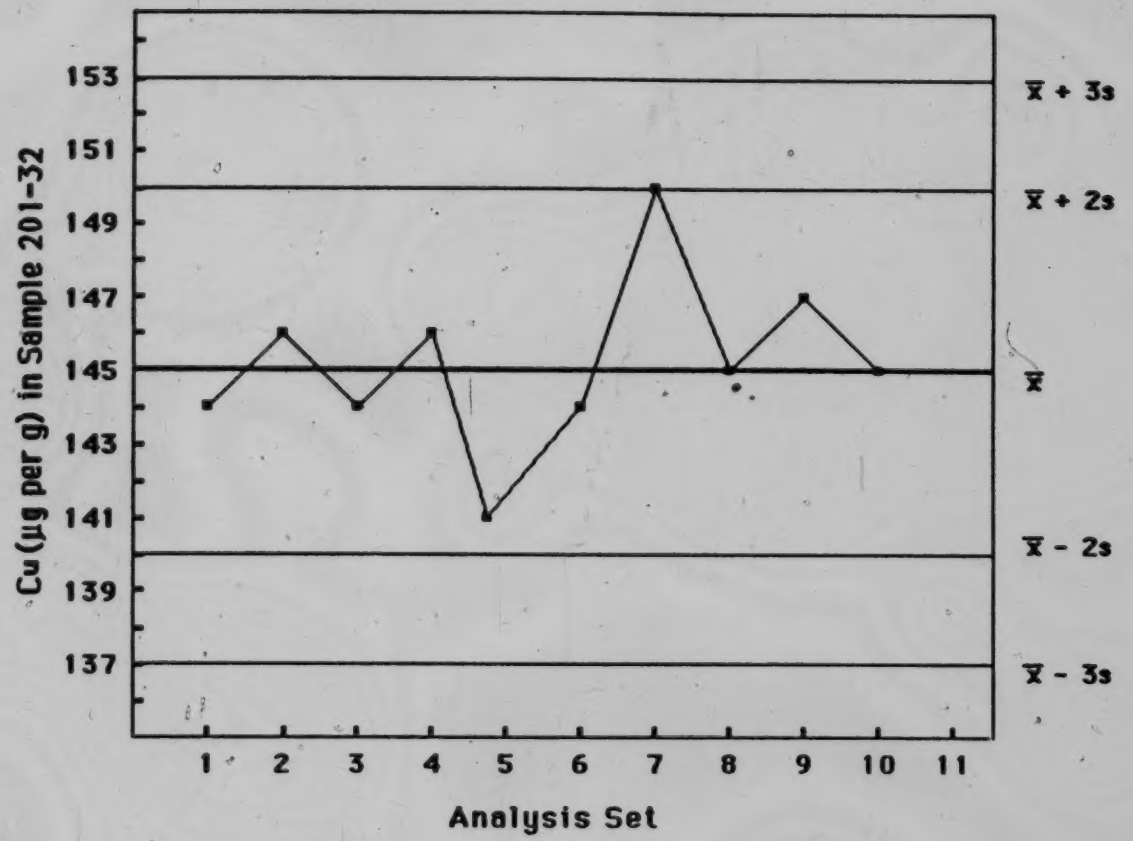

Figure 20. Control chart for copper analyses of soil samples from field 201. Results for analysis sets were obtained on different days (see section 5.3 for details).

Table 25. Values for zinc and copper after microwave digestion of sample 201-32 for quality assurance.

Quality assurance set number	Zinc, µg/g	Copper, µg/g
1	93	144
2	99	146
3	93	145
4	93	146
5	94	141
6	94	144
7	91	150
8	94	145
9	90	147
10	95	145

Each value was run on a separate day, as a control, in conjunction with a set of analyses of other samples. Based on 23 determinations, zinc was found to be 94 ± 3 µg/g. Copper was found to be 145 ± 3 µg/g for sample 201-32.

should be taken if one value falls outside these limits. Since none of the results obtained for 201-32 fell outside the limits shown in Figures 19 and 20, it can be concluded that results obtained for zinc and copper in samples taken from field 201 are in statistical control, thus ruling out the likelihood of systematic errors that might have arisen from incomplete dissolution, reagent contamination or other sources of error.

4.2 Trace Zinc and Copper Distribution in Field Number 201

Zinc and copper results for 52 soil samples from field 201 are presented in Table 26. The spatial variability of these results is shown in Figures 21 and 22, and the statistical [illegible] in Table 27. The results in Table 27 show clearly that in these soil analyses, the largest contribution to the overall error comes from the sampling uncertainty. Also, standard deviation and range values indicate a greater spatial variability of Cu compared to Zn in field 201. This is attributed to the uniform addition of $ZnCl_2$ powder during fertilizer application. The naturally occurring copper in the soil seems to be distributed in a southeast trend in the field. There is a general decrease in copper content from value 149 to 145 in Figure 22. Only one out of 22 samples

Table 26. Overall zinc and copper concentration values in soil samples from field number 201.

Sample	Zinc, µg/g	Copper, µg/g
201-01	96 ± 1	135 ± 2
201-02	95 ± 1	134 ± 1
201-03	89 ± 4	132 ± 1
201-04	97 ± 0	139 ± 1
201-05	102 ± 2	135 ± 4
201-06	97 ± 1	119 ± 1
201-07	93 ± 1	132 ± 0
201-08	82 ± 1	119 ± 1
208-09	96	129
201-10	100 ± 4	116 ± 1
201-11	101 ± 1	124 ± 1
201-12	95 ± 1	114 ± 1
201-13	94 ± 1	107 ± 0
201-14	88 ± 2	118 ± 2
201-15	90 ± 2	119 ± 0
201-16	88 ± 0	116 ± 2
201-17	83 ± 0	119
201-18	99	123
201-19	96 ± 1	140 ± 0
201-20	95 ± 1	128 ± 1
201-21	88 ± 4	134 ± 0
201-22	100 ± 1	125 ± 1
201-23	94 ± 1	116 ± 3
201-24	94	128
201-25	115	137
201-26	98 ± 1	133 ± 1
201-27	94 ± 1	133 ± 0

(Continued)

Table 26. (Continued)

Sample	Zinc, µg/g	Copper, µg/g
201-28	100 ± 1	136 ± 4
201-29	104 ± 1	169 ± 1
201-30	104 ± 1	152 ± 1
201-31	91 ± 1	138 ± 7
201-32	94 ± 3	145 ± 3
201-33	84 ± 1	131 ± 2
201-34	94 ± 0	206 ± 2
201-35	102 ± 2	205 ± 0
201-36	89	202
201-37	92	143
201-38	81	174
201-39	79	168
201-40	86	189
201-41	98	163
201-42	86	156
201-43	94	140
201-44	87	139
201-45	82	136
201-46	81	131
201-47	100	153
201-48	97	150
201-49	111	136
201-50	96	142
201-51	92	141
201-52	92	158

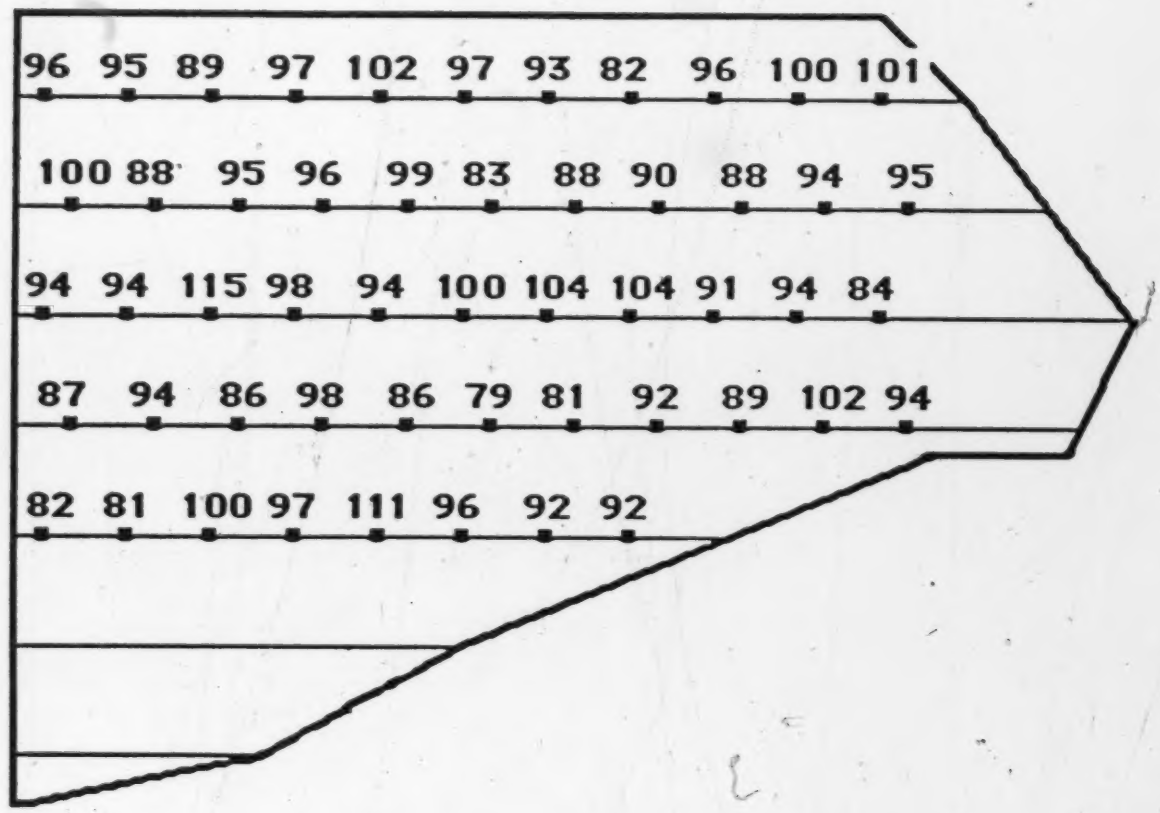

Figure 21. Distribution pattern of zinc in field number 201.

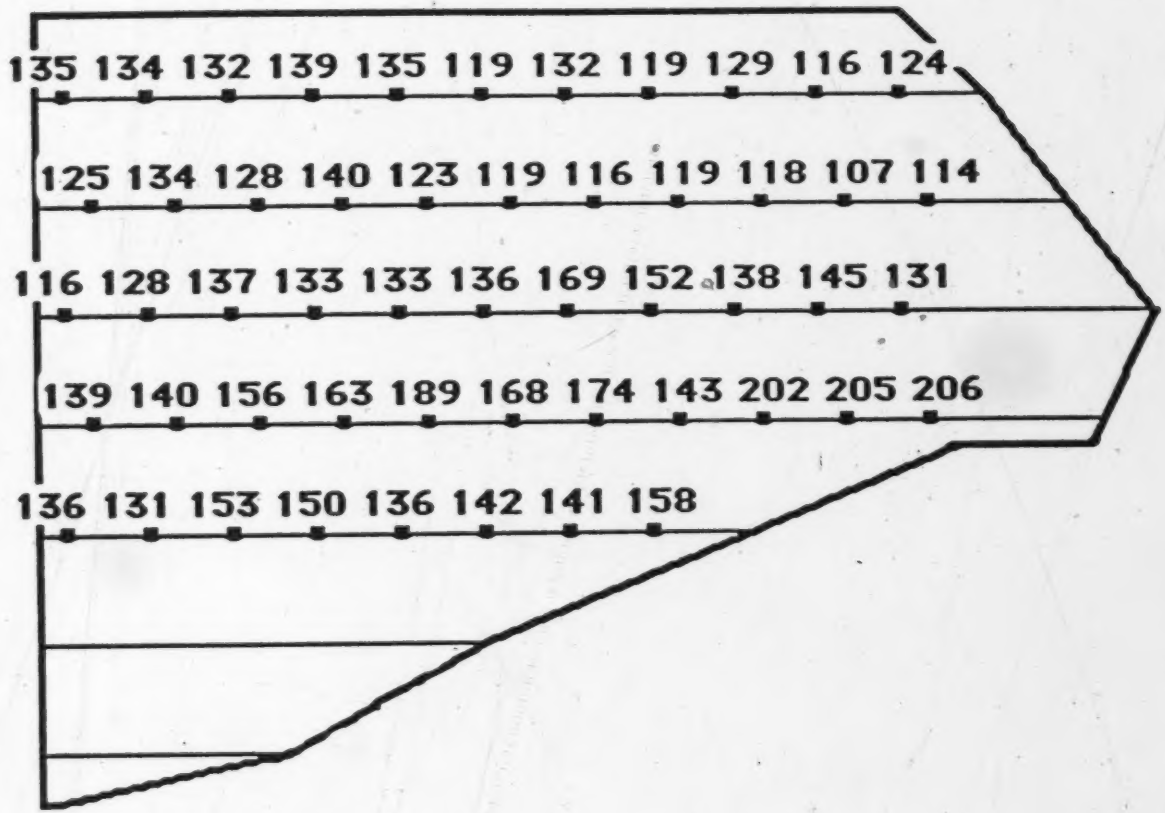

Figure 22. Distribution pattern of copper in field number 201.

Table 27. Statistical summary of zinc and copper concentrations of 52 samples taken from field number 201.

Element	$\bar{x}$, µg/g	Range, µg/g	Standard deviation, µg/g	Relative standard deviation, µg/g
Copper	140.5	206 - 107	22.9	16.3
Zinc	93.8	115 - 79	7.4	7.9

falls at or above the average value in laterals 148 and 149, 3 out of 11 in the middle lateral, and 15 out of 19 samples in laterals 145 and 146. Even though the field slope is downward towards the canal at the bottom of field 201 (see Figures 1 and 2), this cannot be accepted as the reason for the trend in copper results because samples 34 through 36 in Figure 22 have the highest concentrations in the field, yet they are at a higher elevation than samples 43 through 47.

4.3 Testing for Normality of Zinc and Copper Distributions in Field Number 201

Since one of the requirements for application of the student's t-test is a Gaussian distribution of the sought-for subastance, it was necessary to perform tests for a Gaussian distribution of zinc and copper over the entire population to which the equation is applied. Among the several statistical methods that can be used for this test, the Kolmogorov-Smirnov approach [64,65] was chosen for the data set shown in Table 26. The Kolmogorov-Smirnov test involves comparing the cumulative frequency curve of the data set to be tested (observed set) with that of the corresponding hypothetical distribution. This was done by ranking the data in order of increasing size and calculating the cumulative relative observed and the

cumulative relative expected frequencies at each data point. The test-statistic was then obtained by finding the maximum difference between data point frequencies and comparing this value to tabulated values at the 95% confidence level [66]. The maximum difference of 0.089 for zinc was found to be significantly smaller than the tabulated value of 0.185. That of copper (0.190) was larger than the tabulated value but when the "hot spot" values at sampling points 34 through 36 were omitted, the remaining 49 samples gave positive results for the Kolmogorov-Smirnov test. Therefore these points were removed from the data set for further t-test studies.

4.4 Estimates of Increment Number Using the Student's t-test Equation

(a) Evaluation of uncertainties due to sampling

The overall standard deviation values shown in Table 27 incorporate all the errors discussed in Chapter IV, Sections 3.4 and 3.5. The error due to sampling alone, s_s, which must be used in equation (8), can be separately evaluted by rearranging equation (1) to give

$$s_s = (s^2_{aa+vc+ss+s} - s^2_{aa} - s^2_{ss} - s^2_{vc})^{1/2} \qquad (22)$$

For zinc, $s_{aa+vc+ss+s}$ is 7.4 µg/g. Taking s_{ss}, s_{vc} and

s_{aa} to be 3, 1 and 2 µg/g respectively, s_s for zinc becomes $\sqrt{40.8}$. For copper, the overall standard deviation, $s_{aa+vc+s+ss}$, based on 49 samples which tested positive to Gaussian distribution, is 16.9. Since s_{ss}, s_{aa} and s_{vc} are the same as those for zinc, s_s for copper becomes $\sqrt{271.6}$.

(b) Validity of the student's t-test equation for zinc

If the precision requirement is such that the mean estimate of n measurements be within the population standard deviation s_s, the minimum number of samples that will satisfy this precision requirement is obtained by setting the precision level, $\mu-\bar{x}$ (in equation 8) equal to s_s. This reduces the equation to

$$n = t^2 \qquad (23)$$

At the 95% confidence level, the value of t for 52 observations is 2.01 and the initial estimate of n is therefore equal to 4. Iteration to a constant value of n yields 6 as the predicted number of increments required for a precision less than or equal to the sampling uncertainty. To test the validity of this estimate, a table of random numbers [58] was used to select 50 groups

of six samples each. If the prediction is correct, then the difference between the mean of each group of six samples and the true mean, 93.8 µg/g, is not expected to exceed ±6.4 µg/g 95% of the time. Results for this test are shown in Table 28. Out of 50 trials, the precision limit was exceeded only twice, hence the test was successful 96% of the time. This is in good agreement with the predicted probability level of 0.05, so the student's t-test equation gives valid estimates of n for zinc.

(c) Validity of the student's t-test equation for copper

Equation (23) was used to predict the number of samples to be taken in order for the mean estimate of n measurements to be within the sampling standard deviation for copper in field 201. Thus if n equals six, the student's t-test equation predicts that mean estimates fall within ±16.5 µg/g of the population mean of 136.6 µg/g. Table 29 shows the results obtained when the test was carried out on 50 groups of six samples each. The precision limit was exceeded only twice, which is satisfactory at the 95% confidence level.

4.5 Practical Considerations: Future Sampling Protocols

Two approaches to the practical application of the

Table 28. Results for 50 randomly selected groups of six samples used to test the validity of the student's t-test equation for zinc.

Group	Values for zinc, µg/g						$\bar{x}_{Zn}$, µg/g	d, µg/g[1]
1	92	90	87	96	100	86	91.8	2.0
2	83	82	86	115	92	94	92.0	1.8
3	96	100	83	115	96	94	97.3	3.5
4	100	90	89	98	97	94	94.7	0.9
5	94	84	115	97	96	94	96.7	2.9
6	89	93	88	94	100	104	94.7	0.9
7	84	89	96	81	90	82	88.7	5.1
8	115	87	102	96	94	96	98.3	4.5
9	97	81	95	102	94	87	92.7	1.1
10	82	88	100	84	104	115	95.5	1.7
11	88	94	88	89	97	115	95.2	1.4
12	99	94	89	115	92	90	96.8	3.0
13	100	92	94	89	97	94	94.3	0.5
14	95	96	83	104	95	96	94.8	1.0
15	88	94	94	95	81	94	91.0	2.8
16	98	94	89	94	83	98	92.7	1.1
17	92	101	98	100	98	86	95.8	2.0
18	101	100	94	104	92	99	98.3	4.5
19	88	95	86	96	81	101	91.2	2.6
20	94	82	101	88	91	100	92.7	1.1
21	81	89	88	95	94	88	89.2	4.6
22	98	97	94	92	94	81	92.7	1.1
23	94	87	100	79	86	94	90.0	3.8
24	94	93	86	96	96	100	94.2	0.4
25	94	84	100	82	94	102	92.7	1.1
26	96	102	100	96	100	97	98.5	4.7

(Continued)

Table 28. (Continued)

Group	Values for zinc, µg/g						$\bar{x}_{Zn}$, µg/g	d, µg/g[1]
27	81	94	98	92	102	96	93.8	0.0
28	90	100	94	115	91	100	98.3	4.5
29	88	111	92	95	97	82	94.2	0.4
30	94	90	93	79	96	94	91.0	2.8
31	94	86	84	86	111	79	90.0	3.8
32	96	94	100	115	104	99	101.3	7.5[2]
33	94	94	88	91	88	104	93.2	0.6
34	102	86	88	111	92	87	94.3	0.5
35	92	89	86	95	100	88	91.7	2.1
36	104	89	99	90	111	95	98.0	4.2
37	97	90	93	88	95	94	92.8	1.0
38	88	88	86	111	89	96	93.0	0.8
39	83	95	104	88	97	100	94.5	0.7
40	82	101	96	100	95	101	95.8	2.0
41	88	95	96	95	111	92	96.2	2.4
42	115	82	96	95	104	83	95.8	2.0
43	94	97	82	89	96	100	93.0	0.8
44	87	104	96	101	98	94	96.7	2.9
45	86	98	88	84	90	104	91.7	2.1
46	84	98	81	88	81	88	86.7	7.1[2]
47	111	96	97	89	94	82	94.8	1.0
48	95	95	104	86	94	94	94.6	0.9
49	89	97	84	100	96	81	91.2	2.6
50	90	92	94	84	95	96	91.8	2.0

[1]Refers to the difference between the population mean and the mean based on 6 random samples.

[2]d exceeds the desired precision of 6.4 µg/g.

Table 29. Results for 50 randomly selected groups of six samples used to test the validity of the student's t-test equation for copper.

Group	Values for copper, µg/g						$\bar{x}_{Cu}$, µg/g	d, µg/g[1]
1	131	139	119	125	189	158	143.5	6.9
2	143	124	116	128	168	156	139.2	2.6
3	119	168	143	169	116	139	142.3	5.7
4	135	107	136	142	136	124	130.0	6.6
5	153	140	118	132	131	134	134.7	1.9
6	131	142	140	132	139	119	133.8	2.8
7	123	140	116	116	107	135	122.8	13.8
8	152	136	128	142	131	125	135.7	0.9
9	133	152	128	136	131	116	132.7	3.9
10	133	174	118	135	140	168	144.7	8.1
11	132	137	152	119	123	140	133.8	2.8
12	119	135	133	136	134	144	133.5	3.1
13	142	132	136	140	134	137	136.8	0.2
14	143	134	153	150	189	140	151.5	14.9
15	140	124	119	133	132	135	130.5	6.1
16	116	144	137	158	139	119	135.5	1.1
17	134	136	150	135	138	116	134.8	1.8
18	132	169	107	116	116	133	128.8	7.8
19	134	136	119	140	125	144	139.7	3.1
20	124	136	138	129	189	133	141.5	4.9
21	135	134	139	119	118	140	130.8	5.8
22	137	133	132	116	143	153	135.7	0.9
23	153	119	114	139	119	136	130.0	6.6
24	136	139	133	132	116	158	135.7	0.9
25	144	116	168	118	128	137	135.2	1.4
26	119	128	136	142	125	119	128.2	8.4

(Continued)

Table 29. (Continued)

Group	Values for copper, µg/g						$\bar{x}_{Cu}$, µg/g	d, µg/g[1]
27	119	133	116	140	125	152	130.8	5.8
28	124	136	138	163	116	116	132.2	4.4
29	123	174	168	163	158	189	162.5	25.9[2]
30	114	174	107	189	136	116	139.3	2.7
31	125	189	136	119	133	135	139.5	2.9
32	136	153	140	158	119	134	140.0	3.4
33	139	135	125	156	119	136	135.0	1.6
34	156	137	143	150	116	142	140.7	4.1
35	125	119	137	135	163	116	132.5	4.1
36	119	132	163	150	116	135	135.8	0.8
37	142	139	144	156	116	133	138.3	1.7
38	153	189	158	135	123	134	148.7	12.1
39	141	116	163	123	131	131	134.2	2.4
40	129	128	153	132	189	132	143.8	7.2
41	153	132	116	116	125	119	126.8	9.8
42	189	144	114	107	134	139	137.8	1.2
43	132	189	116	138	118	158	141.8	5.2
44	141	189	144	128	134	119	142.5	5.9
45	119	136	150	152	107	144	134.7	1.9
46	119	156	174	168	136	189	157.0	20.4[2]
47	133	139	136	132	142	125	134.4	2.1
48	139	152	135	124	163	116	138.2	1.6
49	156	163	134	131	119	169	145.3	8.7
50	131	163	131	116	174	118	138.8	2.2

[1]Refers to the difference between the population mean and the mean based on 6 random samples.

[2]d exceeds the desired precision of 16.5 µg/g.

student's t-test equation to field sampling can be made. First, the analyst may wish to find out how many samples would be required to achieve a precision of ±10% for both zinc and copper. For most applications in trace soil analyses, this precision is acceptable. As noted in the previous discussion, the value of n is, to a significant extent, dependent on the variability of copper in the field. For six samples, the precision limit for copper was twice that of zinc. The equation predicts that for a ±10% precision, the number of samples required for zinc is 5. Copper requires 8 samples for the same precision. When a field is sampled, the same increments used for zinc determinations are also used for copper, therefore the number of 100 gram soil samples to be taken from this population to achieve a precision of ±10% in future sampling should be 8. This is a minimum value; if less than 8 samples are taken, the desired precision will not be achieved. On the other hand, cost, effort and time requirements increase, with no special advantage gained, when more than 8 samples are taken.

The second approach to applying the student's t-test equation in field sampling is to consider the sampling scheme presently employed for analysis of soil for macronutrients like potassium, nitrogen and phosphorous at the Simunye Sugar Estate. For a 17 ha field like 201, 3

samples are usually taken from each lateral (see Figure 2). Therefore a total of 15 samples taken from field 201 are composited to obtain average values of N, P and K concentrations in the field. Using this approach, a precision of ±4 µg/g (±4% relative) is predicted for zinc and that for copper is ±9 µg/g (±6% relative) at the 95% confidence level for 15 samples.

5. Conclusions

The occurrence of unusually high or low values at a single site in a population (a "hot spot") can create major sampling problems. Since such sites are not usually predictable, there is no way they can be taken into account in a statistical sampling plan. Often their presence does not present a major problem. In the instance of increased copper values for three samples in one area of field 201 (Section 4.2), for example, no problems are created by the abnormal level found; that is, the level is not so high as to be toxic to sugarcane. On the other hand, if the copper levels in a hot spot were so high as to be toxic, or so low as to induce deficiency, then identification of such spots would become important. The detection of such spots is generally done by systematic grid sampling such as was performed in this

study. This is the best means of protection against not detecting such regions. The fineness of the sampling grid should be determined by the importance assigned to locating such spots. If the hot spot area is large enough to include an appreciable fraction of the field, then the field can be divided into two strata and each stratum treated independently from a sampling and statistical point of view.

In the sampling study presented here, the spatial distribution of both zinc and copper in field 201 was found to be Gaussian if the "hot spot" for copper is not included. For this population the student's t-test equation (8) gave reasonable predictions of the number of sample increments to be taken in future analyses in order to obtain any desired precision within the limits of the acceptable error. When sampling for multielement analysis, the minimum number of increments is dependent on the element which shows the greatest spatial variability.

CHAPTER VI

SUGGESTIONS FOR FUTURE WORK

The importance of sampling in the chemical analysis of soil samples for trace metals cannot be overlooked. This study has shown that regardless of the analytical precision, invalid results can be obtained if proper sampling procedures are not followed. The student's t-test equation used in this study to predict the number of increments to be taken from a given population provides a useful guide to the design of sampling plans for soil analyses. Further studies on the applicability of this equation are suggested. Practical validation, rather than by random number generation as was used here, is recommended. This can be done by analysis of test portions of 100-gram soil samples taken from other fields in a systematic grid pattern. In our opinion, 15 samples taken from 20 hectares could likely be expected to provide reasonable precision of the mean estimates of the population for most trace elements at a minimum cost.

The spatial distribution of other biologically active trace elements can also be studied. Since cobalt, molybdenum, chromium, vanadium, etc., can be determined

with reasonable accuracy by instrumental neutron activation analysis without prior elaborate sample treatment, INAA is recommended for their particular study because of its multielement capabilities and speed of analysis.

Since one of the underlying assumptions of the student's t-test is that of constant sample weight, it would be interesting to study the effect of sample size on the outcome of precision limits compared to those predicted by the equation. The 42 samples collected from field number 202 would be suitable for this purpose since individual sample sizes, 200 g, are twice those of field number 201 on which the number of increments n is based.

Also, the possibility of using Visman's equation in the design of sampling plans could be evaluated. This would require a different procedure for sample collection than was used in the present study. One would obtain adjacent sample pairs of the same weight to calculate values for sampling constants A and B by the intraclass correlation coefficient method discussed in Section 2 or, alternatively, obtain two sets of samples of widely differing sizes in order to obtain the constants by equations (19) and (20). The Visman equation would then be used to calculate the number of samples, n, each weighing w units, required to hold the sampling standard

deviation s_s to a pre-determined level at the desired level of confidence. An advantage of this method is that it does not rely on any specific statistical distribution.

Other aspects of the soil population studied in this work could be investigated. For example, boron levels could be assessed, natural background levels of zinc in untreated fields could be determined and a further investigation into the trend observed in the distribution pattern of copper in field 201 could be done. A further investigation into the capabilities of microwave ovens when used for sample preparation is recommended. Even though valid and reliable results for zinc and copper were obtained when soil samples were digested with HF and HNO_3 in a 700 W microwave oven, a complete dissolution of the soil was not possible. If this digestion procedure is to be extended to other elements as well, then the present microwave scheme needs to be improved since the insoluble refractory mineral residue remaining may contain the element of interest. Since various acid combinations have already been tried with no apparent increase in the extent of dissolution, the use of microwave ovens with maximum power ratings in excess of 1 kW, if available, is suggested. This will necessarily mean that if available, digestion vessels which can withstand high internal pressures and temperatures should be used.

Lastly, the problem of finding standard reference materials to match the samples under investigation as pointed out in Section 3 of this work needs to be considered. For future trace element analysis of agricultural soils in Swaziland, proper validation of the methodologies employed requires that preparation of a reference soil be initiated on a small scale. This will involve collecting a reasonable amount of soil from the sugarcane fields and grinding to a particle size distribution comparable to that obtained by the grinding procedures used in this study. After mixing, the soil would be packed in small bottles for interlaboratory comparison if necessary. Such a reference soil could then be run alongside routine analyses to provide quality assurance on the dissolution and measurement operations.

REFERENCES

1. J. Beaton, W. Nelson, S. Tisdale, Eds., "Soil Fertility and Fertilizers", Chapter 3, Macmillan Publishing Company, New York, 1985.
2. J. Beaton, W. Nelson, S. Tisdale, Eds., "Soil Fertility and Fertilizers", Chapter 9, Macmillan Publishing Company, New York, 1985.
3. P. du Preez, "Trace elements in sugarcane with special reference to Swaziland", unpublished study, South African Sugar Association Experiment Station, 1970.
4. P. du Preez, J. Meyer, R. Wood, Proceedings of the South African Sugar Technologists Association, pp 196-204, 1971.
5. K. Smith, Ed., "Soil Analysis", Marcel Dekker, Inc., New York, 1983.
6. D. Pryanishnikov, Ed., "Agrochemical Methods in the Study of Soils", Academy of Sciences of the USSR, V.V. Dokuchaev Institute of Soil Science, Translated from Russian to English by the Indian National Scientific Documentation Centre, New Delhi, 1969.
7. H. Aubert, M. Pinta, Eds., "Trace Elements in Soils, Vol. 7", Elsevier Scientific Publishing Company, Amsterdam, 1977.

8. P. Minkkinen, Anal. Chm. Acta, **1987**, 196, 237-245.

9. B. Kratochvil, J. Taylor, Chemtech., September 1982, 564-570.

10. D. Cameron, D. Laverty, M. Nyborg, J. Toogood, Can. J. Soil Sci., **1971**, 51, 165-175.

11. L. Calvin, R. Petersen, Eds., "Methods of Soil Analysis", Part I, Chapter 5, 1965.

12. L. Drees, L. Wilding, Soil Sci. Amer. Proc., **1973**, 37, 82-87.

13. L. Holcombe, Radian Corporation, P.O. Box 9948, 8501 MO-Pac Blvd., Austin, TX 78766, 1987, private communication.

14. M. Khan, S. Nortcliff, J. Soil Sci., **1982**, 33, 763-770.

15. A.A. Benedetti-Pichler, "Essentials of Quantitative Analysis", Ronald Press, New York, 1956, pp 309-316.

16. B. Kratochvil, J. Taylor, D. Wallace, Anal. Chem., **1984**, 56, 113R-129R.

17. W. Harris, B. Kratochvil, "An Introduction to Chemical Analysis", Chapter 21, Saunders College Publishing, Philadelphia, 1981.

18. W. Harris, H. Laitinen, "Chemical Analysis", Chapter 27, McGraw-Hill, Inc., New York, 1975.

19. P. Gy, "Sampling of Particulate Materials. Theory and Practice", Elsevier, New York, 1979.

20. J. Visman, J. Mat. Res. Stds., **1969**, 9-13, 51-56, 62-64.

21. B. Kratochvil, D. Wallace, Anal. Chem., **1987**, 59, 226-232.

22. B. Kratochvil, "General Principles of Sampling", Sampling and Calibration for Atmospheric Measurements, ASTM STP 957, J.K. Taylor, Ed., American Society for Testing and Materials, Philadelphia, 1987, pp 5-13.

23. A. McBratney, R. Webster, Soil Science, **1983**, 135, 171-183.

24. A. Dow, D. James, "Source and Degree of Soil Variation in the Field", Washington Agricultural Experimental Station, Bulletin 749, Washington, 1987, pp 1-11.

25. SLOWPOKE II Nuclear Reactor Specifications. Atomic Energy of Canada Limited Comercial Products, IND64, 1976.

26. J. Hilborn, R. Kay, P. Stevens-Guille, Int. J. of Appl. Rad. and Isot., **1973**, 24, 509-518.

27. S. Katz, American Laboratory, June 1985, pp 16-28.

28. A. Comu, J. Leckre, Eds., "Newtron Activation Analysis Tables", Heyden, London, 1974.

29. G. Muecke, Ed., "Short Course in INAA in the Geosciences", Mineralogical Association of Canada, Halifax, May 1980.

30. D. Brune, E. Steinnes, Talanta, 1060, 16, 1326-1329.

31. P. Helmke, R. Koons, Soil Sci. Soc. Am. J., 1978, 42, 237-240.

32. B. Kratochvil, N. Motkosky, R. Thapa, Can. J. Chem., 1985, 63, 2679-2682.

33. J. Duke, B. Kratochvil, D. Ng, Anal. Chem., 1986, 58, 102-108.

34. L. Galinier, L. Zikovsky, J. Radioanal. Chem., 1981, 67, 193-203.

35. R. Cornelis, J. Hoste, J. Versieck, Talanta, 1982, 29, 1029-1034.

36. L. Galinier, G. Kennedy, L. Zikovsky, Can. J. Chem., 1986, 64, 790-792.

37. H. Bauer, G. Christian, J. O'Reilly, Eds., "Instrumetal Analysis", Chapter 19, Allyn and Bacon, Inc., Boston, USA, 1978.

38. W. Slavin, D. Trent, Atomic Absorption Newsletter, Nov-Dec 1986, 4, 351-356.

39. H. Bauer, G. Christian, J. O'Reilly, Eds., Instrumental Analysis", Chapter 10, Allyn and Bacon, Inc., Boston, USA, 1978.

40. D. Skoog, D. West, Eds., "Fundamentals of Analytical Chemistry", Chapters 22 and 25, Holt, Rinehart and Winston, New York, USA, 1976.

41. H. Kahn, J. Chem. Ed., 1966, 41,

42. Analytical Methods for Flame Spectroscopy, Varian, Varian Techtron Pty. Ltd., Springvale, Australia, July 1979, publication no. 85-100009-00.

43. K. Jackson, P. Newman, Analyst (London), 1983, 108, 261-264.

44. M. Berrow, M. Stein, Analyst (London), 1983, 108, 277-285.

45. J. Cansul, T. Fries, P. Lamonthe, Anal. Chem., 1986, 58, 1881-1886.

46. B. Kratochvil, N. Motklsky, Department of Chemistry, University of Alberta, unpublished research.

47. L. Jassie, H. Kingston, Anal. Chem., 1986, 58, 2534-2541.

48. A. Schilt, "Perchloric Acid and Perchlorates", G.F. Smith Chemical Company, Ohio, 1979.

49. A. Abu-Samra, S. Koirtyohann, J. Morris, Anal. Chem., 1975, 47, 1475-1477.

50. R. Nardkarni, Anal. Chim. Acta, 1982, 135, 363-368.

51. P. Anderson, P. Aysola, C. Langford, Anal. Chem., 1987, 59, 1582-1583.

52. S. Berman, S. Nakashima, R. Sturgeon, S. Willie, Analyst (London), 1988, 113, 159-163.

53. L. Fischer, Anal. Chem., 1986, 58, 261-263.

54. L. Fernando, C. Gabrielli, W. Heavener, Anal. Chem., 1986, 58, 511-512.

55. J. Bozic, B. Cousins, W. Flora, F. Smith, Anal. Chim. Acta, 1985, 177, 243-245.

56. U. Jones, Ed., "Fertilizers and Soil Fertility", Chapter 8, Reston Publishing Company, Inc., Virginia, USA, 1982.

57. J. Beaton, W. Nelson, S. Tisdale, Eds., "Soil Fertility and Fertilizers", Macmillan Publishing Company, New York, 1985, pp 317-320.

58. M. Natrella, "Experimental Statistics", National Bureau of Standards Handbook, Section 5, T82-T85, Washington, 1966.

59. J. Beaton, W. Nelson, S. Tisdale, Eds., "Soil Fertility and Fertilizers", Chapter 11, Macmillan Publishing Company, New York, 1985.

60. Varian Spectr AA-20 Manual, Varian Techtron, Australia 85-10009-00.

61. P. Rasmussen, Department of Chemistry, University of Swaziland, unpublished research.

62. B. Kratochvil, N. Motkosky, Department of Chemistry, University of Alberta, unpublished research.

63. W. Harris, B. Kratochvil, "Introduction to Chemical Analysis", Chapter 15, Saunders College Publishing, New York, 1981.

64. J.C. Miller, J.N. Miller, "Statistics for Analytical Chemistry", Chapter 5, Ellis Horwood Limited, West Sussex, England, 1986.

65. F. Rohlf, R. Sokal, "Biometry", Chapter 17, W.H. Freeman and Company, New York, 1969.

66. F. Rohlf, R. Sokal, "Statistical Tables", Table 12, W.H. Freeman and Company, New York, 1969.